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(57) Abstract

Methods for the formation and replication of patterns are disclosed, with objectives including economical microfabrication and nanofabrication, suitable for both low and high volume production. A patterned relief is formed on a surface by various techniques, which is then replicated on daughter surfaces. Methods for the reduction or elimination of defects are included. Various compositions may be patterned to address a wide range of applications. Spatial control over copolymer synthesis, synthesis of patterned molecular monolayers and multilayers, fabrication of microelectronic, microelectromechanical and microfluidic devices may be effected by the methods of the present invention. Various articles of manufacture and uses thereof, including improved methods and means for genome analysis, are further disclosed.

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PATTERN FORMATION, REPLICATION, FABRICATION AND DEVICES THEREBY

Field of the Invention:

The invention relates to the fabrication of two- and three-dimensional patterns and the production of devices thereby; the present invention finds application in the areas of microelectronics, micromechanics, microfluidics, scanning probe microscopy, mass data storage, scientific instrumentation, clinical diagnostics, molecular assembly, and other areas.

Related Art:

Microfabrication and Microelectronics:

The field of integrated microelectronics depends critically upon the generation of predetermined two dimensional patterns. Generally, some lithographic process (e.g. involving actinic radiation) is employed to transfer a pattern from a mask to a resist, which is developed to expose portions of the surface of the underlying substrate. Such exposure permits the patterned etching of the exposed surface, or the patterned diffusion of impurities into regions near said surface. Thus, metal layers may be etched to form wiring patterns and regions of a semiconducting substrate may be doped to form electronic components in an integrated device. Metal layers may also be patterned by lift-off processes involving the local dissolution of a pre-patterned resist underlayer[1], which carries away with it the immediately overlying regions of the metal layer. Such resist masking and modification steps are repeated with different mask patterns and different modifications are performed which spatially

vary according to the pattern formed in said resist. The alignment of mask patterns during each step with respect to all other steps is a critical limit on fabrication resolution. A summary of the basic methods is given is described by Millman[2]. Microfabrication of integrated optics devices relies on similar techniques, as well as further additional techniques, a discussion of which is given by H. Nishihara, M. Haruna and T. Suhara.[3]

Fault Tolerant Circuit Designs

One approach that has been applied in the field of microelectronics to increasing the tolerable physical defect rate arising from a particular fabrication process involves fault tolerant circuit design. This is a category of methods which includes redundancy of functions or components, self-testing or quality checking during the fabrication process, and rerouting of connections between functional blocks to serve functions lost to defects. For example, such methods have been applied to dynamic RAM fabrication, where the memory bit array is divided into a number of blocks, the total capacity of which is larger than the device specification. All blocks are checked for defects or functionality, and where defects are found, the involved blocks are deactivated. Functional blocks are then reconfigured, as needed, to contiguously fill the device address space. Analogous methods have been applied in the design of gate arrays, field programmable gate arrays, memory devices and other microelectronic devices.

Microscale and Nanoscale Lithographic Methods:

Defect Reduction by Resist "Voting":

In U.S. Patent Number 5,308,722, J.L. Nistler has disclosed a method for the reduction of defects in lithographic phase shift masks. The essence of this method is to form a resist pattern, use it to etch the underlying quartz surface only partially, remove said resist pattern and any defects it may include, form a substantially identical pattern of resist on said quartz surface again, partially etch again and remove said substantially identical pattern of resist again, in repetitive cycles. Thus, only etch resistant regions appearing in all resist patterns will be reproduced in the final patterned quartz article at the fully etched depth. Conceptually, this method checks one pattern, which may contain random defects, against other patterns

which may also contain random defects which are not likely to appear at the same location as those random defects in any other pattern. In the case of the phase shifting reticles produced by that invention, only regions etched such that the transmitted light is shifted by 180 degrees exposes the lithographic resist these reticles are used to expose. Thus, correspondence between multiple source patterns is required for a feature to be represented in the final product mask produced therefrom.

Contact Printing of Resist by Lithographic Plate:

In U.S. Patent Number 5,380,620, T. Suzuki and F. Shinozaki teach a method whereby a lithographic plate comprising regions of ink binding and ink repelling regions is contacted with an ink or resist sheet and then contacted with a substrate to which said ink or resist which bound to said ink binding regions of said ink. This method forms lithographic plates by exposing materials similar or identical to those used as resists in conventional microfabrication, such that exposed regions have affinity or repel ink or other liquids, such that a pattern of differential retention of ink or said other liquids may be used to form a pattern of said ink or other liquids on said lithographic plates and then transfer this pattern to the substrate. While this method reduces the extent to which photolithographic equipment is needed for patterning, production of micropatterned articles by this method still requires routine access to such photolithographic equipment for lithographic plate production. Further, primary reliance on differences in affinity of liquids to those regions of said lithographic plate treated to have such affinity compared to the affinity of said liquids to the substrate to be thus imprinted entails that this method will require considerable effort for optimization when applied to new inks or resists to different surfaces.

Photolithography Generated Relief Patterns, Mechanical Transfer Thereof, and Use as Lithographic Plate:

Methods for the production and transfer of relief patterns in polymeric resist materials or variants thereof are reviewed by B. Bednar, J. Kralicek and J. Zachoval.[4] A relief is produced by

selectively exposing regions of a photoresist coated onto a first surface and mechanically transferring either the exposed or non-exposed regions to a polymeric foil second surface by juxtaposing said second surface to the image-exposed polymeric resist and relying on the differential adhesion properties resulting from exposure. Thus, a positive image is produced on one surface while the other surface retains the corresponding negative pattern. Such relief patterns may provide differential wetting or liquid-retention properties differing from those of the underlying surfaces, and thus be used as offset lithographic plates.

Differential Resist and Differential Ion Milling Thereby:

G. Gal has, in U.S. Patent Number 5,310,623, taught a method whereby microlens arrays are formed. In this method, greyscale exposure of a resist material may be used to cause the differential etching (in the preferred case by ion milling) of the underlying substrate, to produce a correspondingly curved surface. (Note that this inventor uses the term replica in a different sense than that used herein.) Greyscale exposure results in a different effective etch protection by the resist, according to the degree of exposure. This may be termed etch-resistance density or depth dependent etching.

Microelectromechanical Systems:

Microfabrication techniques have been applied to multilayered substrates comprising sacrificial layers which permit under-etching. Under-etching permits the fabrication of structures with suspended or overhanging members and freely moving microscale mechanical parts. Such a process may be combined with conventional microfabrication steps or applied to substrates comprising microelectronic devices to integrate both electronic and mechanical components of micron and submicron dimension. Such integrated devices have been termed microelectromechanical systems (MEMS)[5].

Microfabricated Scanning Probe Microscopes and Actuators:

J.J. Yao, N.C. MacDonald and S.C. Arney have described microfabricated actuators, springs and STM instruments[6], and have

taught methods for producing components of these in U.S. Patent Numbers 5,179,499 and 5,235,187. These capacitor actuators have nanometer scale accuracy and high resonant frequencies.

S. Akamine, C. Quate and co-workers[7] have described a different microfabricated STM design relying on bimorph piezoelectric actuators.

Data Storage with Scanning Probe Devices:

The high detection resolution achieved by the various regimes of scanning probe microscopy as well as the ability to modify surfaces and structures thereupon with these instruments has motivated much interest in the use of scanning probe technology for data storage and retrieval. It is anticipated that means such as these will be necessary to surpass the physical limits on recording density in magnetic and far-field optical surface recording technologies.

Recently, T.C. Reiley, L.-S. Fan and H.J. Mamin[8] have demonstrated the recording and readout of data using and AFM based instrument and the polycarbonate coated surface of a transparent disk as a storage medium. Data is recorded in the form of pits on said surface. A laser is used to heat said polycarbonate above the glass transition temperature, and the AFM tip impresses a pit into the locally heated region. The size of the resulting pit corresponds to the apical geometry of said AFM tip. Readout is accomplished by conventional contact-mode AFM detection of said pits. Thus, upon recording of a bit pattern onto such a polymer coated surface, a relief pattern is formed which encodes the corresponding, stored data. These workers have formed pits as small as 100nm across and 10nm deep, yielding an areal storage density of 25 Gb/in^2.

R. Imura, S. Hosaka, et al.[9] have also recently demonstrated a scanning probe technology based data storage method, also using an AFM-like instrument with a gold-coated AFM tip. These workers rely on filed evaporation of metal from said gold-coated AFM tip to form dots (representing data bits) on a silicon surface, with readout again accomplished by conventional contact-mode AFM.

In a somewhat different approach, R.E. Betzig et al., in U.S. Patent Number 5,286,971 and elsewhere[10], teach a method by which data may be stored by employing a medium which undergoes physical transformation upon exposure to light and exposing said medium to light according to a near-field transfer process, such that the region

of said medium exposed is limited by the physical dimensions of the aperture transmitting said light rather than the wavelength of said light, where said physical transformation includes a local change in optical properties and where said local change in optical properties is detectable by a method similar to Near Field Scanning Optical Microscopy (NFSOM). In this method, density advantage is gained compared to the wavelength related density limitations of far-field optical recording methods, with densities as high as 45 Gb/in^2 having been demonstrated .

Scanning Probe Pattern Formation:

Scanning Resist Patterning:

Scanning Tunneling Microscopes (STM)[11,12], Atomic Force Microscopes (AFM) and Near Field Scanning Optical Microscopes (NFSOM)[13] have been used to selectively form patterns in surfaces on the nanometer scale. These include patterns in materials or layers which mask the underlying substrate from the action of etchants when not modified by the microscopic probe used.

Chemical Manipulation and Synthesis with Scanning Probe Microscopes:

W.T. Müller, P.G. Schultz et al.[14] describe a patterning process whereby a surface is adsorbed with molecules comprising a chemical functional group which may be transformed to a second chemical functionality by contact with a metal catalyst, an AFM is used to scan a tip coated with said metal catalyst over areas of said surface which are intended to be transformed, and said chemical functional groups transformed by the resulting, spatially limited catalytic process are then subjected to further chemical reactions which are selective for the catalytic reaction product second chemical functional groups. B.J. McIntyre, M. Salmeron and G.A. Somorjai[15] have similarly effected positional control of metallic surface catalysis using a platinum-rhodium STM tip in an atmospheric-pressure chemical reactor to catalyze the rehydrogenation of molecules comprising alkyne functionalities on (111) platinum surfaces.

M.A. Voelker[16] has proposed a method for the patterning of chemical moieties on surfaces, and the formation of patterns in multicomponent Langmuir-Blodgett (L-B) films according to the interaction of affinity

groups of molecules in said L-B films with complementary affinity moieties on said surfaces. Such a method would entail that the molecules of which said L-B film is composed have good mobility in the as-formed L-B film prior to any polymerization, and that transport permits good segregation of molecular species within said L-B film without any congestion problems or other hindrance to the proper assortment of species to match the pattern on the underlying surface. Because said L-B film is only exposed to said pattern after deposition onto said surface, the mobility of molecular species within said film will be reduced as compared to mobility in the undeposited film, and this will reduce the efficacy of the proposed method. Further, though the proposed method provides for multiple uses of the initial patterned surface, it does not provide for the replication of the L-B layer patterns thus formed except where these comprise multilayers which remain associated together. In other words, this method only provides for the additive accumulation of patterned L-B layers, whether these are then isolated from an initial surface or formed successively in a multilayer molecular based structure.

Microfabrication with Replication:

Integrated optical devices and other microscale optical components have been fabricated by the replication of relief patterns into polymeric materials[17]. The optical properties of these materials and the structures resulting from pattern replication determine device function and characteristics. Replication methods used include injection molding and casting into polymeric, elastiomeric or metallic molds or mold inserts, and hot embossing with reliefs, for example, metal reliefs. Multiple cycles of replication may be performed to yield a large number of reliefs for the rapid mass production of devices, according to the fidelity of replication and acceptable device tolerances.

Over the last decade, methods have been developed to form three dimensional patterns on the micron and sub-micron scales, combining lithography with electroforming, micromolding and mold replication. An original microrelief is formed by the lithographic depth patterning of a relief material, which is then developed to form the desired predetermined structure (first relief). Electroforming is then

performed to yield a metal negative mold insert conforming to the polymer resist structure, and then separated from the original resist. This first generation mold may then be as a mold for the casting or microinjection molding of a second polymeric relief nearly identical to the first relief. The molding or casting process may be repeated with the first generation mold, and the electroforming process may be repeated with second or subsequent generation polymeric relief structures produced with said molds by appropriate methods. Thus, polymeric objects and metallic objects with sub-micron patterns may be economically reproduced. This set of methods, known as LIGA, was developed by E.W. Becker et al.,.[18] These workers reported reproduction of lateral features smaller than 0.1 micron. These methods are particularly useful in the production of devices with microscale features and moving parts. Where the lithographic method employed is synchrotron X-Ray lithography, high aspect ratios may be achieved with resists of up to millimeter depth. More recently, M. Abraham et al.[19] have reported the fabrication of micro-optical systems with LIGA, and further extend these methods by using mold inserts thus produced for hot embossing.

Similarly, M.T. Gale et al.,[20] extend methods from the embossed diffractive foil and compact disk (CD) industries by the replication of an original (e.g. microfabricated) microrelief on the surface of a replication shim through electroforming, followed by replication of this first shim by surface passivation followed again by electroforming. These workers find that the shim replication process occurs with only slight (<2nm) increases in surface roughness per generation, thus enabling the reproduction of nanoscale relief features, with corresponding cost advantages. Related injection molding methods are described by A. Neyer et al.,[21] and R. Klein and A. Neyer.[22]

Micromolding of Ceramics and Resonators Therefrom:

J.A. Bride et al.[23] have shown that micropatterned reliefs may be used to micromold ceramic patterns. Specifically, these workers used a polyimide relief, prepared by reactive ion etching through a Ti mask, was used as a "cookie cutter" which was impressed into a chemically softened ethyl-methacrylate tape comprising powdered

ceramic particles (ceria-zirconia) of average size 0.3 microns. After embossing, the tape was dried, removed from the micromold, heated at 600 degrees centigrade to remove the EMA binder and then sintered at 1500 degrees centigrade. Features as small as 4 microns could be thus produced. These workers have developed this method for the fabrication of piezoelectric ceramics.

Self-Assembling Monolayers and Resists Therefrom:

A paradigmatic example of self-assembling monolayers is provided by the self assembly into monolayers of alkanethiols onto gold surfaces, according to the strong affinity of thiol groups for gold surfaces.

As reviewed by A. Ulman,[24] these self-assembling monolayer systems also include organosilanes and derivatives thereof (e.g. $RSiX_3$, R_2SiX_2 or R_3SiX where R groups may be identical or distinct), which form covalent linkages to surface hydroxyl groups (e.g. formed on surfaces with exposed SiO_2 including native silicon oxides, SnO_2, TiO_2). Note that according to appropriate chemistries, monolayers thus formed may be modified such that the molecules of said monolayers thus formed bear terminal hydroxyl groups, which may then serve as donors for the successive, covalent addition of monolayers, forming crosslinked multilayered structures on said surfaces. Note also that the organic chains of such monolayers may additionally comprise polymerizable or photopolymerizable groups such as alkynes.[25] Further, such monolayers may be used to render surfaces hydrophobic, such that they are protected from a solution with which said surfaces are contacted, such as an aqueous etchant solution.

Simple Methods for Initial Patterning of Monolayer Resists:

Several groups have formed patterns in homogenous self-assembling monolayers (SAMs) formed on metal surfaces or surfaces of metal films, generally gold films, by methods related to milling or grinding. These methods include scraping with sharpened tools[26] to produce the desired pattern as well as applying increased force to an AFM tip during scanning operations or increasing the setpoint current to cause physical contact of an STM tip so said metal surface with increased local forces resulting.

G.M. Whitesides and co-workers[27] have also produced a rapidograph-like micropen for the application of organothiol compounds to specified surface regions, and used this writing instrument to produce organothiol patterns on gold surfaces.

Microcontact Printing of Self-Assembling Monolayer Resists:

G.M. Whitesides and co-workers have disclosed methods for the microcontact printing of compounds which form SAMs onto surfaces coated with evaporated metal films, with a strong emphasis on organothiol compounds used in conjunction with evaporated gold film coated surfaces. These workers have demonstrated that metal regions coated by said monolayers are resistant to wet chemical etch solutions. After such a chemical etch step, the underlying surface is exposed; where said underlying surface is, for example, crystalline silicon, it may be wet etched to produce a relief pattern corresponding to the mirror image of the relief surface used in said printing. Microcontact printing methods used by these workers has been based upon relief patterns replicated in elastiomeric polymer surfaces, generally using (PDMS) elastiomers and reliefs replicated from microfabricated surfaces. These methods are found by these workers to yield a defect rate (of over $1/mm^2$ per mask-etch cycle)[28] which they deem unacceptably high to make these methods competitive with conventional lithographic techniques for the production of microelectronic devices. They speculate that these defects primarily owe to microcrystallites in said gold films, arising from incomplete coverage of said microcrystallites by said organothiol molecules.

Patterns of organothiol SAMs by these methods have reproducible resolution of features smaller than 100nm. Such patterns have also been formed on the surfaces of glass cylinders and fibers, which have been used subsequently to replicate the corresponding pattern on flat surfaces by a rolling technique, analogous to that used in the printing of holograms in metallized polymeric films but on a smaller scale.

Wettability control by Self-Assembling Monolayer Composition and Structures Thereby:

G.M. Whitesides and co-workers have further demonstrated that patterns of such organothiols affect the wettability of the surface regions they coat, and that the presence of a pattern of a first organothiol can mask the adsorption of a second organothiol, such that when a pattern of a first organothiol is disposed upon a gold surface and said gold surface is then contacted with a solution of a second organothiol, said second organothiol forms a SAM only on exposed areas of said gold surface; the resulting surface is thus completely coated with two complementarily patterned organothiol SAMs. Thus, said first organothiol may have a terminal methyl group such that regions with a SAM of said first organothiol composition are wetted well by organic liquids and poorly by aqueous or polar solutions, and said second organothiol may have a polar terminal group, such as a hydroxyl group such that regions with a SAM of said second organothiol are wetted well by aqueous or polar liquids and poorly by organic liquids.

With appropriate patterns thus formed, the patterned wettability of such surfaces has been used to fabricate surface-tension defined structures, such as a microlens array[29] from solutions comprising optically clear polymerizable compounds. Wetting may be conducted, for example, by vapor condensation or dipping into solutions.

Lithographically Patterned Copolymer Synthesis:

S.P.A. Fodor et al.[30] have disclosed methods for the spatially directed synthesis of copolymers including biopolymers such as oligonucleotides and polypeptides. These workers bind initiator chemical groups protected with photolabile protecting groups to a substrate. Spatially masked exposure to appropriate wavelength actinic radiation removes said photolabile protecting groups from exposed regions, where the extent of said exposed regions is delimited by an exposure mask having a desired radiation masking pattern. Said surface is then contacted with a solution of photolabile protecting group protected monomers of desired composition, which react only with the deprotected (photodeprotected) initiator chemical groups located at exposed surface regions. Unbound monomers are then washed. Said masked exposure step, said protected monomer contacting steps and said washing steps are repeated so as to synthesize desired oligomers at desired locations on said surface such that an array of diverse oligomers situated on said surface is produced and the location of an

array element comprising oligomers bears a predetermined relationship to the sequence composition of said oligomer copolymer located at said array element. Such copolymer arrays may be used, depending on the compositions of said copolymer, to detect affinity binding to members of a population of molecules (comprised within the composition of said array) of a sample, which methods are useful to screen antibody specificity, to detect the presence of polynucleotide sequences in a sample or mixture, and for sequencing by hybridization methods.

Other methods for the production of similar copolymer arrays have been demonstrated by others, albeit generally at lesser array element densities.

Array Fabrication by Masking for Materials Screening:

X.-D. Xiang et al.[31] have recently described a method for the screening of materials comprising the repetitive evaporation of different materials onto a surface occluded by a mask, such that an array is formed comprising elements of different composition, which array is then sintered. Array elements thus prepared may then be subjected to screening for desired properties in analogy to the methods of the field of combinatorial chemistry.

Microcontact Patterning of Proteinaceous Arrays on Surfaces:

Whitesides and co-workers[32] have used elastiomeric patterned relief surfaces to produce surfaces with correspondingly patterned deposits of proteins. In this method, a gold surface is patterned with an organothiol, and then exposed to and organothiol comprising terminal hydrophilic moieties are then adsorbed to unpatterned regions. Such a surface is then contacted with a solution of the desired protein or proteins, which adsorb to the hydrophobic surface regions. Such a method does not provide for the formation of patterns of variations of combinations of proteins from one region to the next.

Production of Molecular Multilayers with Monolayer Precision:

Various workers[33,34,35] have described methods for the stepwise addition of monolayers or bilayers to a surface, such that a

multilayer structure comprising a predetermined number of layers of only a few nanometers in thickness may be formed. According to these methods, once a monolayer or bilayer is thus deposited on a surface (including surfaces comprising monolayers, bilayers or multilayers), said monolayer or bilayer remains situated upon said surface.

Synthesis of Sheet-like Polymers:

S.I. Stupp et al.[36] have shown that substantially linear molecules comprising extended linear regions along their length, a polar enantiomeric ordering center and two polymerizable reactive groups of distinct polymerization chemistry at a specific positions along their length spontaneously assemble into layered mesoscale structures which may then be polymerized to yield two dimensional polymers. This work continues the teachings of S.I. Stupp disclosed in U.S. Patent Number 5,229,474.

Terminally Functionalized Rod-like Molecules:

M. Kotera, J.-M. Lenh, and J.-P. Vigneron[37] have disclosed compounds comprising rigid rod molecular segments with single terminal nucleobase moieties. Said compounds self assemble in organic solvents to form supramolecular rods according to well-known Watson-Crick pairing rules. The resulting rods self-organize into mesoscale structures.

Immobilization of Molecules to Scanned Probes:

There have been some successful efforts to derivatize the surface of a scanning probe microscope probe (or tip) with molecules. One set of methods coats the tip surface with gold and then incubates with organic thiol compound to coat the tip to form some functionalized surface.[38,39,40]

Another set of experiments has relied on the strong non-specific association of bovine serum albumin (derivatized with biotin) and the silicon nitride commonly used to produce such microfabricated tips.[41,42]

Similarly, G. Lee et al.[43] have immobilized oligonucleotides to the tips of cantilevers and associated these to complementary oligonucleotides bound to juxtaposed surfaces, and have measured the forces developed as such associations are ruptured by withdrawal of

said cantilever. Here, oligonucleotides were synthesized to comprise a thiol group which was crosslinked to an amine functionalized organosilane derivatized silica AFM tip. Disruption of association of complementary oligonucleotides was shown to be reversible.

Positional Synthesis of Molecules:

D. Eigler has demonstrate the direct manipulation of atoms adsorbed to metal surfaces with a Scanning Tunneling Microscope, and the direct assembly of molecules from said atoms. This work involved nonspecific interactions between said atoms and said surfaces, and said atoms and the STM tip. These experiments were carried out at liquid helium temperatures under ultra-high vacuum conditions, because these conditions were necessary for the stability of the interactions used and the molecules (multimers of $CsCl_2$) thus produced. These atom-by-atom manipulations provide useful insight into scientifically interesting processes and the properties of otherwise inaccessible compounds, but because of the extremity of the conditions required, do not suggest practical methods for the assembly of useful molecular structures from atoms.

K.E. Drexler[44], after R. Feynman, has made elaborate proposals concerning the positional synthesis of nanoscale, three-dimensional structures, generally on an atom-by-atom basis. Practical methods for such synthesis have yet to be presented, though C. Musgrave has undertaken theoretical analyses of factors related to the assembly of diamondoid structures from reactive carbon species under mechanical or positional control and forces exerted on reactants thereby (referred to as mechanosynthesis).

Objects of the Invention:

It is an object of the present invention to reduce the capital costs of microscale and submicron patterning in the fields of microelectronics, micromechanics and MEMS. It is a further object to reduce the minimum feature size below that attainable with visible light in certain embodiments. It is an object of further embodiments to facilitate the inexpensive prototyping and low-volume production of such devices. It is a yet further object of the present invention to provide for the inexpensive production of miniaturized scanned probe devices. It is a further object of the present invention to provide

for the used of said miniaturized scanned probe devices in the parallel positional synthesis of complex molecules and supramolecular assemblages.

It is an object of the present invention to extend the applicability of patterning methods to molecular monolayers, multilayers and polymers therefrom.

It is an object of the present invention to provide an inexpensive method for the synthesis of complex copolymer arrays without extensive use of lithography. It is a further object to extend the methods of use of such complex copolymer arrays to provide enhanced capabilities and refinement of discrimination.

Summary of the Invention:

The present invention employs either contact printing , contact molding, or devices produced thereby, in combination with pattern replication, and applies these to the fabrication of numerous devices. An initial or master relief pattern or pattern of surface composition is produced by prior art methods or by methods of the present invention. This master is replicated one or more times by methods provided within the present invention, permitting rapid expansion of template size and number of templates . Final generation templates are used in device production. Macroscale to nanoscale features may be achieved by these methods, including within the same fabrication process and article of manufacture. Preferred embodiments include the generation of masking patterns for etch control and diffusion barrier formation in semiconductor fabrication, microfluidics fabrication, MEMS fabrication and the like, patterned chemical synthesis and copolymer synthesis, the formation of patterned molecular monolayers and multilayers, replication of chemical patterns, fabrication of microstructured and nanostructured materials and combinations of any of the foregoing.

Description of the Invention:

Throughout this disclosure, it will be assumed that patterned relief surfaces are composed of materials that are either unreactive to any chemical species which are applied to them or may be pretreated to render them similarly unreactive, or otherwise that any reactivity

to said chemical species which are applied to said patterned relief surfaces occurs or may be caused to occur to only a negligible extent. It is also assumed, as is obvious to those skilled in the relevant arts, that precautions must be taken to avoid microscopic contaminants, which cause defects at micron and submicron scales; such precautions include ultrafiltration of solutions, and protection from atmospheric contaminants. It is further noted that it will be obvious to those skilled in the relevant arts that adhesion layers, resist layers and sacrificial layers may be used as needed without departing from the scope of the present invention.

All references are incorporated by citation.

Resist Molding:

As an alternative to the patterning methods described by Whitesides et al. whereby patterns are defined by the transfer an organothiol to a gold surface and formation of a SAM thereby, a resist or masking resist pattern may be formed upon a surface by molding an appropriate material in the desired pattern on said surface or by analogous techniques such as casting, injection molding, or alternatively by a method comprising the steps of sheet stamping followed by a brief pre-etch to eliminate protecting material in reduced thickness regions formed by said sheet stamping step.

In this process, a first relief pattern is replicated in a convenient material, such as by the polymerization of a prepolymer (preferably an elastiomer) on said surface, casting a plastic, polymeric or other appropriate solidifiable liquid material with said first relief surface, plating with one or more metals by known art techniques, or, stamping into a melted material such as is practiced in hot-foil technology and CD replication, to define a mold. A mold is thus formed with a pattern defined by said first relief pattern, and has surface features corresponding to the negative pattern formed by said first relief (defined as the positive pattern). (Note that alternatively, said first relief pattern may be fabricated so as to itself be the negative pattern, from which further negative patterns are generated in even numbered replication generations.) Said first relief pattern is fabricated either by prior art means or by methods disclosed within the present invention. Relief feature height (or depth) is predefined so as to permit reliable pattern formation,

reliable cast or molded material self-cohesion and substrate surface adhesion, and also reliable release of said cast or molded material from said from said negative replicated relief. Optimal feature sizes will vary according to the materials and materials combinations chosen, and specifics of any particular embodiment of this category of methods.

Said initial pattern may be used repetitively to form the opposite pattern on a replica surface such that high quality first generation replicas are formed in a quantity increasing arithmetically with each replication cycle. First generation replicas formed by said first relief pattern may be used in turn to generate subsequent generation replicas, with multiplicative increase in replica quantity per replication generation. Thus tradeoffs will exist between the quantity of replicas formed in a particular length of time and the quality of replicas thus formed. High fidelity replication methods, such as are used in the fabrication of integrated optical devices and components thus permit the rapid production of replicas; negative replicas are then used to mold a resist pattern onto a surface.

In a preferred case, a prototype master is used repetitively to create negative replicas neighboring each other on a first generation replica surface, which is then in turn used repetitively to create replicas of the entire adjacent group of first generation replicas, again in an adjacent configuration. Thus, a single pattern is repeated an increasing number of times on the surface generated by each generation of replication. Thus, advantageous tradeoffs between relief life, rapidity of pattern production, and pattern fidelity may be attained according to the demands (e.g. time, volume, quality) of production. Calculations relating these will be obvious from the necessary empirical data and production constraints.

Several different procedures are available for the formation of a resist pattern on a surface with such a mold. These methods exclude masking material from regions contacted by the highest extent of said negative replicated relief, which, if of elastiomeric composition, will conform to the contours of the substrate surface when juxtaposed to said surface under sufficient normal force. Resist material is not excluded from recessed regions of said negative replicated relief, and allowed to harden, polymerize, cure or is exposed to appropriate illumination to photo-cure, as appropriate, after which said negative

replicated relief is evenly removed in a manner sufficiently gentle to ensure that the resulting resist pattern is not damaged.

Note that molded resist layers may favorably be formed on the surfaces of adhesion layers which have first been deposited or coated onto said substrate surface without departing from the above aspect of the present invention. Here, for example, an ultra-thin, uniform adhesion layer which is sensitive to an etching solution or procedure comprising polymerizable chemical functional groups may first be situated upon a substrate, which is then, for example, coated uniformly with a photopolymerizable polymeric resist, which is then patterned under pressure with a clear relief surface and then photopolymerized by exposure to appropriate wavelength light, with the resulting polymeric resist material covalently linked to said polymerizable chemical functional groups of said adhesion layer. Said adhesion layer is chosen to not protect the underlying substrate surface from etching treatments or procedures, and is formed at a thickness sufficiently small to prevent the significant under-etch of the overlying resist material (due to the small cross-sectional area and correspondingly small diffusion rate through such an area).

Note that such an underlayer may, when possessed of sufficient elasticity, alternatively permit the use of a metal relief replica as said negative replicated relief because said elasticity serves the same function as the elasticity of an elastiomeric mold, i.e. ensures that juxtaposed surfaces subjected to normal forces conform to each other such that liquids do not significantly interpenetrate between the contacting areas or said juxtaposed surfaces; as above, resist precursor material is excluded from areas of said adhesion layer contacted by said negative. In this case, the article being patterned conforms to the relief rather than the reverse situation with elastiomeric negative replicated relief molds.

A yet further variation involves the use of metal or other rugged reliefs as "cookie-cutters" which mechanically exclude a softened resist precursor such as a melt or sol-gel from surface regions underlying outermost extensions of the applied relief structure.

Fabrication by Layered Molding of Patterned Article Material and Sacrificial Material:

Techniques such as those described above for molding of structures using relief masters may be used recursively where each repetition adds another patterned layer (i.e. a portion of a layer in a predetermined pattern which may additionally have surface features or height contours) of material for the desired final article or another patterned layer of sacrificial material useful in the production of unsupported or overhanging structures.

Here, sacrificial materials may include waxes which are melted away at elevated temperatures or dissolved in solvents such as alcohols. These would most favorably be used as sacrificial material with polymers that polymerize in aqueous solution or other solutions in which said wax is substantially insoluble.

Sacrificial material may also be used to flatten intermediate surfaces of article material as an article is being built up by this method. Here, article material is patterned by molding, casting or stamping, and when hardened (if hardening is required,) the article under fabrication is juxtaposingly contacted to a flat surface which is coated with softened or liquefied sacrificial material (where as necessary a release agent is used between said flat surface and said sacrificial material to facilitate removal of said flat surface after said flattened material has set or hardened.) This method is advantageous where an article is conveniently built up in layers with substantially flat borders.

Note that article material is preferably chosen such that where article precursor material (such as a prepolymer) is overlaid onto polymerized or hardened article material, chemical crosslinks will form between these regions of two such juxtaposed layers. This will be the case, for example, for polymers where polymerization occurs via side-groups or where there is an excess of one of the two chemical functionalities which are involved in polymerization, such that a plurality of unreacted functional groups are available on the surface of such article material after polymerization of that portion of article material has proceeded to completion.

Complex mechanical and micromechanical systems with considerable depth and complexity along the depth axis may thus be produced by repetitions of such a procedure, with removal of the sacrificial material used being performed by appropriate treatment after the final layer of article material has been added.

With this method, prototyping requires the production of initial reliefs corresponding to each pattern of article material, which may be used in turn to pattern such material.

Resist Defect Reduction Methods:

For microfabrication embodiments of the present invention, variations of the methods of Whitesides et al. are used. The unacceptably high defect densities which have dissuaded these workers from continuing efforts in applying this methods to commercial microfabrication and microelectronics fabrication may be prevented in a number of ways such that an acceptably low defect density may be achieved. By such methods, the simplicity, economy and high resolution capabilities of microcontact printing may favorably compete with deep-UV lithography and other more conventional high resolution patterning techniques.

i. Metal Film Annealing:

The defects encountered by Whitesides and co-workers are attributed by these workers to crystallites arising in the gold film deposition process. It has been determined[45] that an acetylene flame annealing step yields atomically flat terraces extending hundreds of nanometers. STM shows these surfaces to be better coated with an organothiol derivative (dimethylaminoethanethiol) than the unannealed film. M.D. Ward and co-workers[46] have previously found that annealing a gold wire with a hydrogen flame yields atomically flat terraces extending over hundreds of microns. Because atomically flat surfaces are better substrates for the formation of self-assembling monolayers, and because crystallites are reduced or eliminated by such thermal annealing, thermal annealing steps will thus reduce the occurrence of defects when added, before the formation of the patterned organothiol SAM, to the procedure of Whitesides and co-workers. It is, of course, necessary that the annealed film be permitted to cool (i.e. thermally equilibrate with the temperature of the relief and applied solution) before application of the SAM forming material, because thermal contraction will otherwise distort the desired pattern.

ii. Multilayer Vetoing:

Defects which are small and occur approximately randomly, as is the case as the gold-organothiol resist masking method of Whitesides et al., may be prevented from appearing in the etched final product by a vetoing method which bears analogy to the reticle voting method described in U.S. Patent Number 5,308,722 by J.L. Nistler. An elastiomeric relief may be prepared by related art methods or as otherwise disclosed in the present invention, and then coated with an organothiol solution. In the present case, a thin layer of gold is deposited by suitable means onto a substrate. This is then thoroughly cleaned and contacted with said patterned relief surface which has been coated with an organothiol solution. The pattern of said relief are thus transferred, in the form of a SAM of said organothiol, to the surface of said gold film, in a first printing step. The patterned surface thus produced is then coated again with gold, forming a second gold film layer, and again patterned in a second printing step with the same relief surface coated either with the same or a different organothiol compound solution. The uncoated regions of said second gold film layer are then etched by appropriate solutions, such as strong acids, such that the underlying substrate is exposed in these unprotected regions and thus susceptible to etching and impurity diffusion. Where said substrate is composed of semiconductive materials, electronic, microelectronic, MEMS and other devices may be produced through processes comprising the above method. For defects that occur due to incomplete SAM formation in the contacted regions, whether due to defects arising during the formation of said gold film layers or in the monolayer formation process (i.e. incomplete coverage of a satisfactory gold surface region) which are not due to large surface protrusions, such a multilayer patterning method will prevent defects from leading to the improper exposure of substrate surfaces after said etch step because only defects in the topmost layer organothiol or topmost layer gold film will permit exposure of the next underlying organothiol layer on the next gold layer. Where defect occurrences are independent events from layer to layer, defects are reduced in such a process as the power of the number of layers used; for the above two layer process, the probability of a defect occurring in a unit of area is squared. Assuming a defect 1 square micron in size occurs on average once, randomly, in an area of $1mm^2$, the relative defect area of 10^{-6} yields a defect rate per two-layer

mask of 10^{-12}, which should be tolerably low for most applications. Such a defect rate may be reduced further by three or more repetition of the above coating and printing steps.

Of course, such methods will require correspondingly refined alignment between multiple masking steps, which may be ensured by more accurately machined alignment means which are designed to fasten to the reference points (e.g. drilled holes of precise location and diameter, three or more per different resist used in a pattern generation procedure) on relief negatives with high positional repeatability. Such considerations and methods for accomplishing the required alignment will be obvious to those skilled in the arts of microfabrication. Note that for purposes of the present invention, reliefs may favorably incorporate features which facilitate alignment between successively applied relief patterns, such as features, favorably placed at borders which generate Moiré patterns under appropriate illumination and imaging (which may include electron microscopy) due to the overlap of patterns in said substrate and said negative relief. Note further that the methods of the present aspect of the present invention very directly also permit accurate tactile alignment, where said features comprise interlocking reliefs which only fit together when said relief and said substrate are in precise registry with each other. Such tactile alignment methods may thus permit repeatable accuracy to within a few nanometers with appropriately designed patterns and sufficiently resilient materials. Higher tolerances will require increasingly careful choice of materials and control and uniformity of temperature to prevent significant distortions due to thermal expansion. As will be obvious, such alignment methods may be used with other aspects and embodiments of the present invention.

iii. Initiator Polymerization:

A further approach to defect reduction is applicable to defects arising from incomplete SAM formation in relief contacted regions. Here, the chemical composition of said SAM is chosen such that the exposed surface of said SAM comprises chemical functional groups which serve as initiators for some polymerizable material. Note that compounds which do not form SAMs may also be used, provided these are reliably deposited on said substrate and do not diffuse; compounds

used need only suitably form patterned initiator compound regions. A quantity of material of said chemical composition (for example, dissolved in a first solution) is applied in sufficient but not excessive quantity to a desired patterned surface, which is then contacted with a substrate to transfer said material of said chemical composition to said surface in a pattern determined by the pattern of said patterned surface. Said surface is then washed to remove any excess reactants or reagents. Said material of said desired chemical composition, now situated in a patterned manner on said surface, is then contacted with a second solution comprising monomer reactant species which are capable of reacting with said chemical functional groups which serve as initiators. Said second solution preferably contains said monomer reactant species at low concentrations such that molecules of said monomer reactant species are more likely to react with said chemical functional groups which serve as initiators, or oligomers or polymers therefrom, rather than with other said molecules of said monomer reactant species. The limit on the concentration of said monomer reactant species are those posed by non-specific polymerization, i.e. significant polymerization of said monomer reactant species into molecules not in communication with said chemical functional groups which serve as initiators. The term significant here refers to the quality of the overlayers thus produced and the non-coating of substrate regions not comprising said chemical functional group which serves as initiators, i.e. reduction of resolution in non-resist coated regions. These polymerizations are thus preferably conducted so as to primarily yield solid phase immobilized polymer products. Said second solution may further comprise multimeric monomers or other crosslinking reagents which serve to cause branching of polymer chains during a polymerization process. Conditions of reaction, the concentration of said monomer reactant species, and the proportion or surface density of said chemical functional groups are chosen such that the polymer coating formed will be substantially limited to the regions of said surface originally contacted by said patterned surface, i.e. not extending far from any of said chemical functional groups which serve as initiators. For example, monomeric species may be added at a limiting quantity, or at a concentration sufficiently low that excessive polymerization does not occur within some conveniently short period of time. This method

of surface coating patterning followed by coating thickening (which may be termed overlayer formation) serves to enclose any small resist layer defects without significantly compromising patterning resolution. Such enclosure reduces or prevents transport of etching agents to defect sites and etching products from said defect sites. As defect size increases, this method requires that overlayer thickness be increased correspondingly to ensure enclosure or encapsulation of defects of said defect size.

For example, said chemical composition of said SAM may be chosen to comprise an alkanethiol moiety and a methacrylate moiety. A solution comprising a quantity of said chemical composition is applied to a gold film situated on a substrate, such that a SAM is formed according to the pattern of said patterned surface. Said gold film and substrate is then washed to remove any excess unbound material. Limiting quantities of methacrylate and any desired polymerization accelerators, as well as a desired ration of polyfunctional or branched methacrylate species are added, and overlayer polymerization is permitted to occur to a desired extent, corresponding to the defect size to be thus eliminated. Unreacted materials are then washed away with appropriate solvents that do not degrade said overlayer. The surface thus treated is thus patterned with a resist layer of corresponding thickness which does not have pinhole defects or other small defects smaller than some critical size.

Note that the methods of this aspect of the present invention are readily applied to patterned thin film coatings which are not SAM based, provided that appropriate chemical functional groups which serve as initiators for some polymerizable material may be incorporated. Note further that any chemical functionality which may be modified (e.g. deprotected, activated, or reacted with some other initiator bearing chemical species) such that it may later serve as an initiator for polymerization reactions is comprehended within the spirit of this aspect of the present invention.

Resist Formation by Wettability Control:

The methods of G.M. Whitesides and co-workers involving the wetting of patterned surface regions, which these workers have used to form microlens arrays from polymer solutions, may also be applied to the elimination of small defects. In this approach, patterns of resist

are formed by first patterning a SAM which is preferentially wet by a resist monomer solution or precursor solution on a surface which is poorly wet by said solution. Said patterning is accomplished by one or more of the methods described herein, such as microcontact printing, and the patterned said surface is then contacted with a resist precursor solution, which is then withdrawn. Portions of said resist precursor solution retained on said surface are then caused or permitted to polymerize, such that a developed resist layer is formed. Thus, defects related to incomplete masking may be eliminated by their enclosure by the overlying resist precursor solution and hence the resulting developed resist. Etch steps are then performed as in conventional microfabrication practice.

In the case of pattern which cause self-organization of said resist precursor solution, and hence predetermined desired geometries of said developed resist layer three dimensional structure, resist depth-dependent etching, in analogy to that described by G. Gal in U.S. Patent Number 5,310,623, may be performed, for example by reactive ion etching, to replicate the self-assembled resist structure in the underlying solid substrate material.

Patterned Metallization:

Metallization, for example, for the formation of electrical contacts, may be accomplished according to the patterning methods of the present invention and corresponding extensions of related art methods, as well as by the application of related art methods to articles produced by the methods of the present invention (e.g. formation of alkanethiol patterns by microcontact printing on gold films, employing, as necessary, the defect reduction methods disclosed herein).

Molded materials such as those used above for resist purposes may, with appropriate solvents, be used as lift-off layers which eliminate any metallic film situated on the surface of said molded materials upon exposure of the surface comprising these to said appropriate solvent. Patterns of said metallic film remain in regions which were not covered by said molded materials.

As will be obvious, formation of metal films may be accomplished by vacuum evaporation of the corresponding metallic material onto the surface of the article under production. Alternatively, such lift-off

patterning may be applied to thin electroformed (e.g. electrodeposited or electroless plated) metallization layers.

Alternatively, metallization may be accomplished by the hot embossing of a first surface with a metallized polymeric foil with the metallized surface juxtaposed to said first surface, under sufficient pressure and at sufficient temperature to ensure good electrical contact with exposed regions of said first surface, followed by dissolution of said polymeric foil and underlying lift-off regions. Such transferred films may be subjected to brief electrodeposition steps to ensure good electrical contact, though care must be taken to avoid the formation of short circuits.

Microelectronics Device Fabrication:

The foregoing fabrication methods comprising the use of relief patterns used to pattern resist or masking layers may be used to control the etching and doping (impurities diffusion) of semiconductor materials and overlying dielectric layers, as well as the formation of metallization layers for electrical interconnection. These methods serve all of the patterning functions required in microelectronics fabrication.

Note further that the above described resist molding and casting methods may similarly be applied to conductive polymers compositions such as those comprising polyparaphenylene and derivatives thereof, polypyrrole and derivatives thereof, polythiophene and derivatives thereof, or other such conducting or electroactive polymers, so as to directly form polymeric devices rather than resist patterns. These materials and devices based thereupon may further be combined with more conventional microelectronic devices fabricated by the methods of the present invention.

Initial relief patterns may be formed in, for example, glass or silicon surfaces by known art methods including photolithography, or may be formed by appropriate scanning probe based patterning methods of related art, or combinations thereof. For example, a glass surface may be coated with a gold film, upon which an organothiol SAM is formed, which is then patterned by STM or field emission mode STM, followed by an acid etch to remove exposed gold regions and a base etch to remove thus exposed glass regions.

It is important to note that a large patterned area may be formed by the replication of a smaller pattern from an initial relief across said large patterned area, as, for example, a single integrated electronic device pattern layer (e.g. corresponding to a mask layer) repeated many times across the surface area corresponding to a wafer diameter. Thus, the replication methods of the present invention may be applied both to expand the number of occurrences of a pattern on a single surface as well as the number of surfaces thus patterned.

Spatially Controlled Copolymer Synthesis:

A relief patterned surface, preferably of elastiomeric composition, may be used to spatially control the pattern of addition of monomer or macromonomer reactants to a subset of appropriate chemical reactive sites on a substrate surface. Here, instead of the addition of single species to surface regions according to the pattern of an elastiomer surface, as done by Whitesides et al., reactants are added to regions of the surface of said substrate according to the pattern of said patterned surface, permitted or caused to react, and unreacted or unbound species are then washed away. Generally, said substrate will comprise one or more substantially flat surface regions and be prepared by the reaction or adsorption of some chemical species to said surface such that chemical species having desired reactivities are bound to said substrate, which is thus capable of being reacted with desired reactant species. Said relief patterned surface is partially or completely coated with a solution of a desired reactant, or said desired reactant is deposited from vapor phase onto said relief patterned surface. In the former case of reactants dissolved in said a solution, said a solution may further comprise inert compounds which increase the viscosity of said solutions. Said relief patterned surface is then contacted, in a contacting step, with said substrate to transfer said desired reactant to one or more predetermined regions of said substrate. Thus, said desired reactant is applied only to the desired regions, according to the predetermined pattern of said relief patterned surface.

The control of precise copolymer sequence thus synthesized is favorably achieved, for example, through the use of protection/deprotection or activation chemistries to effect step control over reactant addition to the monomer or copolymer species

with which said desired reactants are reacted. Thus, reactants added will generally comprise a protecting group or an activatable reactive group, which serve to prevent multiple monomer or macromonomer addition to the same molecule. Through successive cycles comprising the steps of contacting said substrate with desired patterned surfaces coated with desired reactant species, to permit spatial control over reactant addition, followed by substrate washing steps, and steps preparing product copolymer species for subsequent reactions (e.g. deprotection steps or activation steps by chemical or physical treatments of said substrate surface according to the polymerization chemistries and step control chemistries employed), complete spatial control over synthesized copolymer sequence may be achieved. During successive cycles, different patterned relief surfaces are used at different steps, and may optionally be shifted in location from one step to the next; similarly, different desired reactant species may be used during different cycles. This is in analogy to multiple masking steps in related art microfabrication, and directly related to the multiple masking methods of other aspects of the present invention.

By such methods, arrays of copolymers of predetermined sequence, such as oligonucleotides, polypeptides, biomimetic copolymers and non-biological copolymers may be produced at a resolution equal to that with which a relief master may be fabricated. Patterns having features many times smaller than one micron are thus achievable, and correspondingly high densities (such as 10^8 to 10^{10} array elements per cm^2) may be achieved without extensive use of costly lithographic equipment. Such methods thus carry several advantages over the methods of S.P.A. Fodor et al. in that protection/deprotection chemistries (and the corresponding reactant compounds or protected/deactivated) need not be limited to those responsive to actinic radiation or other energetic beams. In other words, reactants useful for ordinary (e.g. not light controlled) stepwise syntheses are useful with these methods. Further, these methods are suitable for the synthesis of copolymer of compositions which might be or are desired to be sensitive to photochemical or other energy induced degradation.

In a second variation, such a said patterned surface may be used to control the spatially controlled activation or deprotection of copolymers or copolymer precursors, such that only the copolymers or

copolymer precursors located at predetermined regions of said surface are prepared for subsequent reaction with a monomer or macromonomer, which is thereby added only to said copolymers or copolymer precursors located at said predetermined regions. Here, a relief is for example coated with solutions comprising deprotection or activation reagents at sufficient concentrations (and preferably also inert constituents increasing the viscosity of said solutions) and contacted with said surface to effect the spatially controlled deprotection of the product precursor copolymers situated thereupon. A solution comprising said monomer or macromonomer is then contacted with said substrate such that these species may react with any available corresponding reactive groups.

The methods of this aspect of the present invention thus permit the synthesis of a potentially large array of diverse copolymer species situated on a surface according to a predetermined pattern, such that composition of the copolymers at a particular region of said surface correspond in a known fashion to the location of said region. Thus, where activities are uniquely displayed by particular regions of said surface, the copolymer sequence composition responsible for said activities may directly be deduced from information about the spatial location of said particular regions.

Note that this method may be used to synthesize or otherwise target copolymers, affinity groups or other molecular functionalities or decorations to predefined regions of the surfaces of microreliefs, micromolded components and components of micromechanical or microelectromechanical systems. In these instances, the reliefs used for pattern formation are produced from master structures which are identical to the molded surfaces in regions to which said reliefs are to contact said microreliefs, said micromolded components, etc., and thus situate reactant monomers or other reactive compounds, but which have surfaces or surface features of increased height at regions not to be targeted by said reactant monomers or other reactive compounds. In other words, the reliefs used to target reactants to predetermined regions of contoured surfaces conform to the geometry of those surfaces in regions to be targeted or modified, but do not contact regions to not be affected due to clearance within said relief that prevents contact of the surface of said relief with regions of said contoured surfaces not targeted.

In the case of copolymer synthesis on preferably flat surfaces, reliefs may be mounted on a roller apparatus, in analogy to arrangements used in holograph stamping processes, to facilitate rapid patterned deposition of reactants.

Note that for variations relying on selective contacting of said substrate with reactants rather than with deprotection reagents, rinse steps may favorably be performed with reagents that react with unreacted monomer reactants to inactivate said unreacted reactants such that these will not, upon washing, react with species bound to regions onto which said unreacted reactants were not contacted by said relief. For example, in the case of phosphoramidite oligonucleotide synthesis chemistries, alcoholic solutions may be used to inactivate and wash away unreacted phosphoramidite monomers, which will react with the hydroxyls of alcohol molecules.

Example Synthesis Procedure:

A first surface, such as a glass surface, is patterned with a first alkylsilane compound comprising an extended methylene or polyethylene glycol linker, or other substantially linear linker chosen to be well solvated or wetted by oligonucleotide synthesis solvents or chosen to be readily modified so as to be well solvated or wetted by oligonucleotide synthesis solvent, and an esterified terminal hydroxyl group by use of an elastiomeric relief with a square grid pattern such that square regions of said first surface are modified by said first alkylsilane compound with distinct unmodified borders (which are hydroxylated) remaining. Untreated regions of said surface may then optionally be permitted to react with a second alkylsilane compound comprising a terminal functionality which will be poorly wetted by the solvents containing oligonucleotide synthesis reactant monomers. Said first surface is then treated with appropriate reagents such as bases to hydrolyze the terminal ester group of said first alkylsilane and thus deprotect the terminal hydroxyl group. A solution of 3' or 5' protected oligonucleotide synthesis reactant monomers of one nucleobase type is then coated onto a second surface, onto which an elastiomeric relief is impressed to transfer some of said reactant to the surface of high regions of said elastiomeric relief (with raised square regions corresponding to a subset of the raised square regions of the relief used to form the regular array of said first

alkylsilane), or the entire surface of said elastiomeric relief is directly coated with a solution of said reactant monomer. Said relief with reactant situated on its surface is then contacted, in an orientation and position which aligns the array element features of said relief with those of the previously formed grid of the alkylsilane on said first surface, with said first surface, such that said reactant is contacted with the exposed hydroxyls of said first alkylsilane compound, and reactions occur linking this nucleotide monomer with the hydroxyls of said first alkylsilane compounds in the affected array elements. Such a process is carried out with different relief patterns and the remaining three nucleotide monomers, such that all array elements experience the positionally controlled addition of exactly one of the four nucleotide precursor species. Note that in this example, said first surface regions which may have optionally been treated to be poorly wetted by said reactant solutions will serve as borders impeding the spreading of these reactants beyond targeted array elements; said well solvated linker moieties are drawn into the solutions with which they are contacted, even where these are situated on surfaces which are poorly wetted by said solutions with which they are contacted (in which case undesirable spreading of reactants to inappropriate surface regions is prevented while synthesis is not frustrated). Said first surface is then thoroughly rinsed. Capping reagents (such as acylating reagents) may optionally be applied to all of said first surface to prevent any further elongation of unreacted hydroxyl groups. Other standard oligonucleotide synthesis steps, such as oxidation, may then be performed as required by the particular chemistry used. The entire array is then treated with reagents to deprotect the reactant monomers added during the previous cycle (e.g. submerged in a solution comprising trichloroacetic acid in the case of trityl protecting groups, exposing a terminal hydroxyl group which is the target for further reactants). The second base of all array elements is then added, in a nucleotide addition cycle comprising the coating of four distinct reliefs (for example, together selecting all array elements but sharing none) with four distinct reactants, and four contacting steps as above, followed by appropriate steps according to the chemistries used, as above. Such cycles are repeated until the array thus synthesized substantially consists of oligonucleotides of the desired length (i.e. n cycles total to produce

an array of n-mers) and desired element sequence according to position within said array. Finally, steps deprotecting nucleobase moieties and, where applicable, deprotecting the phosphate backbone, are performed to yield the desired final molecular structure, as in conventional oligonucleotide synthesis methods, excepting support removal steps. Information regarding the configuration of raised array elements, the reactant and cycle with which they are associated, is preserved such that position of an array element corresponds in a known way with the sequence of reliefs and reactants used to synthesize the oligonucleotides therein, which in turn thus have the corresponding sequence composition. The density (number of array elements and hence distinct sequences per unit area) of arrays thus synthesized may rival or exceed that possible with light directed patterning methods. An important advantage of this method is that conventional oligonucleotide synthesis reagents may be used.

Alternative copolymer array patterned synthesis methods:

The relief forming methods described above may be used to produce a stencil capable of masking surface regions from exposure to reactants. Here, a substrate is first coated uniformly with a sacrificial layer, which is hardened or set. Then, an elastiomer precursor is applied to the surface of said sacrificial layer and then molded by a negative relief. Alternatively, analogous casting or injection molding may be utilized, or embossing of a polymeric film with brief post-etching of the embossed film may be used. Said sacrificial layer is then dissolved to free the patterned elastiomeric stencil.

Such a stencil is used in combination with an array of microfabricated columns, said columns being of uniform height, and with a pitch smaller than the smallest stencil feature. Said stencil may either be combined with said array of microfabricated columns into one stenciling body, or said stencil and said columns may merely be fused together. In either case, said stencil is juxtaposed to the substrate which is to be subjected to patterned copolymer synthesis, and said array of microfabricated columns is pressed onto said stencil, such that said columns force the stencil surface into good masking contact with said substrate. Solutions containing reactants (e.g. deprotection reagents or monomer reactants) are then caused to

flow through said array of microfabricated columns, such that areas not masked by said stencil are exposed to said solutions. Before said stencil is released from said substrate, thorough wash steps are performed to remove residual reactants or reagents. Differently patterned stencils are used in predetermines sequence with different monomer types to produce the desired diverse copolymer array with sequence correlated to location in a predetermined manner.

In the case that said array microfabricated columns is to form one body with said stencil, these may be joined together, e.g. by coating the upper surface of said stencil with an adhesive or crosslinking agent which will react with both the material of said columns and the material of said stencil. Compounds used for such joining must be selected so as to be inert to the reagents and solvents which will be passed over them.

Note that said array of microfabricated columns may favorably be produced by LIGA methods or other replication methods including those of the present invention, and may favorably be composed of metals, e.g. formed by electrodeposition or electroless plating methods.

Uses of copolymer array decorated articles:

Surfaces prepared according to the above aspect of the present invention may be used in a variety of ways including for sequencing by hybridization (where said copolymers are oligonucleotides or oligonucleotide analogs), sequence confirmation by hybridization, for the screening of small peptides and the determination of affinity interactions (as described by Fodor et al., referenced elsewhere herein), for the detection or determination of antibodies, in clinical medical assays, and in the screening of organic material for desired properties according to known methods in combinatorial chemistry.

Sequencing by hybridization, as well as other techniques involving binding determination, may favorably be performed by contacting samples with such an array, where sample molecules binding to said array is monitored by any of several known art techniques, while physical or chemical conditions are changed and binding is recorded (e.g. by capture of signals detected by such monitoring and recorded electronically) as a function of conditions. In a preferred instance of binding determination with variation of physical and/or chemical conditions, the condition dependent properties of sets of array

elements (i.e. copolymer sequences) of interest are determined by observation of condition dependent binding of known samples, and information thus obtained is used to further refine the discrimination of correct (i.e. most highly matched) or most specific binding from incorrect or relaxed specificity binding.

For example, in this way, difficulties in sequencing by hybridization methods arising from differences in the conditions for optimal binding specificity which correspond to individual array elements (oligonucleotides), which frustrate attempts to find globally optimal binding conditions without sacrificing the specificity of many or some of the array elements in an array comprising large numbers of distinct oligonucleotide elements, may be overcome. Here, conditions which may be varied include temperature, ionic strength, divalent cation concentration, presence and/or concentration of tetramethylamonium chloride, presence and/or concentration of denaturant compounds such as formamide or dimethylformamide, pH, and other factors known to affect the hybridization specificity and stringency of oligonucleotides or polynucleotides, may be varied individually or in combination. For example, the binding, and also dissociation of a particular sequence with the complementary n-mer oligonucleotide will occur at a characteristic temperature (i.e. having a characteristic T_m melting temperature at which half of all homoduplex complexes dissociate), and this property verifies the correctness of base pairing.

Array Detection of Rare Molecular Species:

Such arrays may be used to detect very low quantities, approaching single molecules, of complementary molecules in samples by a few distinct methods.

According to the first method, a target array is designed in a hierarchial manner such that as monomers or subunits are added, they are added to subsets of regions to which the previous monomers or subunits were added. For example, an oligonucleotide array is produce by forming first square regions, subdivided into four square regions each containing one of adenosine, guanosine, cytosine or thymidine nucleotides (e.g. through a set of four steps, each step adding one nucleotide to one subdivision region). During the successive synthesis steps, identical squares with sides one half that of those

of the preceding step and area one quarter that of the preceding step are formed, again subdivided into four square regions each containing one of the four nucleotides, preferably in the same relative configuration as that of the preceding set of steps, are added, such that one subdivision square of the previous step is thus subdivided into four subdivisions. Such a hierarchial subdivision process comprising n repetitions of the above sets of four steps (one for each nucleotide moiety) is repeated to produce an array of 4^n n-mers, or desired subsets thereof. For purposes of detecting a rare molecular species, a set of arrays of oligonucleotides of increasing length, e.g. {(n-m), (n-m+1)...(n-1), n}-mers in the case of single nucleotide increments, is produced as above. This set of arrays is designed such that each successive array in the series is in the mirror image of the preceding array of said series, with at least one further synthesis cycle (set of synthesis steps) further subdividing the regions opposed to the smallest array elements of the preceding array. According to the expected relative abundance of the species in a sample to be detected, said sample is bound first to the array consisting of the smallest number of elements. Thus the sample is divided such that each oligonucleotide targets a region of some subset of molecules in said sample. Hybridization to such a lower density array by rare species will be more accurate under appropriate conditions in that fewer array elements of larger area present more molecular targets and fewer degrees of freedom. Thus the kinetic limitation on binding depends mainly on the diffusional transit time from across said array. After a sample is bound to such a lower density first array, it is juxtaposed to a further subdivided mirror image second array, with only a small gap between these arrays, which is filled with an appropriate buffer. Conditions are changed to denature the association of sample molecules with said first array, and then to binding conditions sufficiently stringent that said sample molecules will favorably bind to their target oligonucleotides on said second array in preference to forming the lower energy association with their shorter targets on said first array. This process results in successive subdivision of said sample, such that each subdivision step successively narrows the volume which must be traversed by a sample molecule in order to find its correct binding target, and

further correspondingly reduces the chances that a molecule will find an incorrect target.

An analogous method within the scope of this first method may be applied where an array is designed, as above, such that array elements are similar (but not identical) in sequence to most of their nearest neighbors, such that spatial proximity is related to ordered sequence similarity. This similarity may be used to serve a similar "narrowing down" function accomplished above by juxtaposition of hierarchially related mirror image arrays. In the present case, conditions affecting binding stringency are varied such that after a sample is first applied, only a few contiguous base pairs are sufficient for binding. The regions bound by a sample molecule comprising a specific sequence may either be within the contiguous region comprising many array elements having m of the total n monomers in common (starting from the first variable nonomer added during the first cycle of array synthesis), or with m contiguous monomers offset from the copolymer terminal from which synthesis began, in which case, for each class of array elements with said m monomers in common, contiguous regions of elements comprising said m monomer sequence complementary to said specific sequence, where classes are distinguished according to the number of monomers by which said m monomer sequence is offset from said first variable monomer, become increasingly small and increasingly distant from each other. This results in reduced dwell time in incorrect regions (incorrect with respect to the exactly complementary n-mer target) but in the presentation of larger contiguous targets by regions comprising the n-mer target. In other words, said specific sequence binds to the correct region comprising the n-mer target and similar n-mer sequences according to an equilibrium expression where the proportion of said specific sequences bound to correct regions is proportionate to the concentration of unbound said specific sequences times the relative area of said correct region (in direct analogy to the concentration of m-mers in a conventional equilibrium constant expression). Conditions are then made gradually more stringent, such that more associations between contiguous base pairs are demanded for binding. Stringency (i.e. the conditions affecting stringency) may be oscillated in an increasing trend, such that sample molecules are most likely to home in on their final target oligonucleotides. Those sample molecules which do not

initially bind sufficiently near their targets will enjoy increased diffusional mobility as those sample molecules which have bound to said array are less likely to impede their diffusional transport through the sample solution, e.g. after they no longer bind m-mer comprising elements further from their correct targets under more stringent conditions.

Generalizing this method to any copolymer with sequence dependent binding properties, physical and chemical conditions may be varied to examine the characteristics of, or detect the presence of, binding activities corresponding to respective sets of conditions, or spatial or temporal gradients thereof.

Note that designations above of terminal m-mers are chosen for clarity rather than limitation. The essence of the forgoing description concerns the hierarchially imposed proximity of array elements with similarity which generally decreases with increasing distance of elements in said array. While an m-mer may occur in many array elements in many distinct regions of said array, it remains the case that the m-mers in phase with the target n-mer sequence are grouped together in one contiguous region comprising said target n-mer, regardless of the ordering of synthesis cycles with respect to such hierarchiality.

A second method, applicable to polynucleotide samples, involves the amplification of rare species, or sequence fragments thereof. In this case, established art methods such as the polymerase chain reaction (e.g. with PCR primers flanking the diverse target sequence) or the ligase chain reaction (e.g. for an 8-mer array, involving all 4-mer oligos under conditions sufficiently stringent to preclude any mismatch within said 4-mer sequences for binding) are used to amplify the sample target sequences, which may be a diverse sequence in a conserved sequence context. Thus, small quantities of molecular species within heterogeneous samples may be amplified to a sufficient extent that detection of binding to an oligonucleotide array is conveniently accomplished. Amplification reagents (e.g. primers or nucleotides for PCR or oligonucleotides for LCR) may be labeled, for example, with one or more affinity moieties (e.g. biotin, digoxigenin, etc.,) or one or more fluorescent dye moieties (e.g. Texas red, rhodamine, fluoroescien, etc.,) or one or more other reporter groups or portions. For these amplification methods, amplification will

produce a geometrically increasing quantity of sequences matching the amplified sequences but also a related quantity (which may be biased by such known art methods as asymmetric PCR)

Spatially Controlled Macromolecular Collocalization:

Similar operations as used to synthesize copolymers on a surface in a spatially predetermined manner described above may similarly be used to collocalize multiple distinct macromolecule or macromolecule types to predetermined surface regions. For example, such methods may be used to generate a very large number of positionally predetermined combinations, shapes and/or patterns of large numbers of adhesion proteins which may be used , for example, to test the effect on cell adhesion, cell motility, cell shape or cell physiology in biological and clinical assays, as shown by Whitesides and co-workers for patterns consisting of a single protein species, as well as in other biotechnological uses, including the interfacing of cells to microelectronic devices and sensors. These methods may avail the protein patterning methods disclosed by Whitesides and co-workers[47,48], or may avail other immobilization chemistries. The combination of protein patterning with combinatorial methods thus enabled permits the study of competing influences when different pattern, relative abundances, and different combinations coexist. Note that in these cases, the binding of the macromolecules, which may or may not be macromonomers, may be according to affinity interaction or less specific weak interactions, i.e. need not be covalent binding.

The present aspect of this invention thus depends on the use of a relief pattern comprising raised surface elements to contact a solution containing one or more protein or polypeptide to portions of a first surface to which said protein or polypeptide will adsorb, wherein contact only occurs between said raised surface elements of said relief pattern and said first surface, such that a pattern of said protein or polypeptide is deposited in the pattern corresponding to said raised surface elements onto said first surface, wherein such patterning steps are repeated to yield deposits of plural different proteins or polypeptides at different regions of said first surface, such that different combinations result in a spatially predetermined manner.

Patterned Combinatorial Materials Deposition:

As reviewed above, X.-D. Xiang et al.[49] have recently described a method for the production of arrays of combinations of material, which are then screened to discover combinations with useful properties. The patterning methods described herein may be extended to the production of such arrays but further permitting the rapid, selective deposition of materials which are not conveniently evaporated or without the use of evaporation equipment. Here, a suspension of very fine particles of a material is applied to the surface of a wettable relief, which is preferably of elastiomeric composition. The liquid layer of said suspension on said relief is sufficiently thick that an adequate quantity of suspended material per unit area is obtained on the surface of said relief. This will depend on the viscosity and surface energy of the liquid used. Such a suspension coated relief is then juxtaposingly contacted to a porous flat surface, which may be the surface of a filter. Depending on the composition of the material of said flat surface, capillary action may draw the particles in said suspension onto said flat surface, as in slip-casting. Material transfer of this kind will only occur in areas of contact with said relief, i.e. those raised areas of said relief. Where the composition of the material of said flat surface does not suitably permit capillary action to be relied upon with the solution used to suspend a material, said flat surface must be that of a porous filter, so that negative pressure may be used to draw the suspension solution through said filter, depositing suspended material onto said filter surface. After repeated cycles of deposition of at least two but preferably more different suspensions of different materials, in a pattern determined by the patterned relief used, a patterned array of different materials mixtures is yielded. This may then, for example, be subjected to annealing steps. Thus, large numbers of different combinations of materials may be produced in a convenient format for testing and characterization, including from materials which are not suitably evaporated.

Template Patterned Synthesis of Sheet-like Complexes and Copolymers:

The formation of chemical patterns on surfaces permits the use of such chemically patterned surfaces as templates for the production of patterned molecular complexes and copolymers, which themselves may be used as templates for complementarily patterned molecular complexes and copolymers. This aspect of the present invention is analogous with other aspects of the present invention in that spatially patterned surfaces may be replicated from an initial pattern and thereafter from each other. In the present aspect, patterned compositions of sheet-like molecules serve as *molecular* templates for the definition of patterns in thus complementarily patterned sheet-like molecules in a molecular replication process analogous to the polymerase chain reaction invented by K. Mullis for the amplification of polynucleotide molecules.

The foregoing pattern replication methods of the present invention and those found in related art, as well as scanning probe based patterning methods[50,51,52] may be used to perform the patterned chemical derivatization of surfaces. As shown by examples from related art, such modifications may cause the preferential binding of molecular or macromolecular species to predetermined regions of said surfaces, which are demarcated by said patterned chemical derivatization. Such surface modifications may be used, for example with the methods of G.M. Whitesides et. al. or those of W.T. Müller, P.G. Schultz, et al., to bind specific first affinity groups or species to predetermined regions of said surface. Said affinity groups or species are chosen so as to bind affinity derivatized layer forming molecules comprising a second set of affinity groups or species which are in communication with a layer forming precursor moiety (such as a polymethylene chain, or rigid rod oligomer or polymer). The affinity moiety of said second set of affinity groups is preferably located at one terminus of said affinity derivatized layer forming molecules, which are preferably substantially linear in structure. Said affinity derivatized layer forming molecules preferably but not necessarily further comprise a moiety of third affinity group or species, which is preferably located at the terminus opposite that at which said affinity moiety of said second set of affinity groups is located; said affinity derivatized layer forming molecules may also comprise one or more (preferably two or more) distinct reactive, cross-linkable or photopolymerizable chemical

functional groups, situated along said layer forming molecules such that each of said distinct reactive, cross-linkable or photopolymerizable chemical functional groups occurs at a particular position along the length of said layer forming molecules, and may, after a layered structure has been formed by association of moieties selected from said second set of affinity groups with said first affinity groups pattered on said surface, be caused to react to form a covalently linked network joining said layer forming molecules. Said first affinity groups and said second affinity groups are preferably chosen such that the interaction between them is sufficiently strong and specific under a first set of conditions but is substantially weakened or neutralized under a second set of conditions; thus, under said first said of conditions, layer forming molecules assemble at surfaces comprising regions of said first affinity group in patterns corresponding to the pattern of said regions, while said second set of conditions serves to separate layers comprising said affinity groups chosen from among said second set of affinity groups from surfaces (including surfaces of layers) comprising said first affinity groups. Said third affinity group or species is chosen such that it does not bind to either of said first affinity groups or said second set of affinity groups, but will bind to affinity groups selected from a fourth set of affinity groups, which in turn will not bind to said first affinity groups, other affinity groups selected from said fourth set of affinity groups, nor to said second affinity groups (i.e. said first affinity groups and affinity groups of said second set of affinity groups bind only to each other and not to identical affinity groups; said affinity groups chosen from said third set of affinity groups and said affinity groups chosen from said fourth set of affinity groups bind only to each other and not to themselves; e.g. sets of affinity groups may each consist of one of adenine, thymine, cytosine or guanosine nucleobases). The composition of said surface in unpatterned (or differently derivatized regions) is chosen to not bind said affinity derivatized layer forming molecules. After a pattern of said first affinity groups is formed on said surface, a solution containing said affinity derivatized layer forming molecules is contacted with said surface under appropriate conditions and for sufficient time to permit the binding of said affinity derivatized layer forming molecules to all available sites on said surface.

Unbound species are washed from said surface. Thus, said layer forming molecules comprising affinity groups from said second set of affinity groups are bound to said surface in a pattern replicating the pattern of said first affinity groups on said surface. These may then be cross-linked via said reactive groups, for example by the addition of suitable cross-linking agents, photopolymerized, or caused to react together by establishing appropriate physical or chemical conditions such that a covalent network is formed. Note that said affinity group chosen from said third set of affinity groups may be initially caged or protected to suppress the formation of associations until a deprotection step is performed. In this instance, affinity groups chosen from said fourth set of affinity group may be reacted with regions of said surface not containing said first affinity group, in direct analogy to the complementarily patterned two component monolayers disclosed by G.M. Whitesides and co-workers, formed by patterning a first component monolayer and then permitting a second component compound to self-assemble into a monolayer on remaining uncoated regions. Alternatively, patterns composed of domains of multiple distinct affinity moieties may be formed by multiple relief contacting steps, or by multiple spatially selective activation steps (examples include: light directed, for photodeprotectable or photoactivatable compound derivated surfaces; scanning probe controlled surface catalysis such as the methods of W.T. Müller, P.G. Schultz et al., or of B.J. McIntyre, M. Salmeron and G.A. Somorjai, or other scanning probe based patterning methods capable of directing the adsorption or reaction of molecules comprising the desired affinity moieties to the selected regions of patterned surfaces; and, the replication methods of related art or of the present invention, e.g. involving microcontact printing). Multiple distinct molecular species comprising multiple distinct affinity moiety types or classes and types may be patterned onto the same surface by repetitive cycles of patterned activation or patterned masking followed by deposition of the respective compound (e.g. by contacting a solution of said respective compound to said patterned activated or patterned masked surface) such that deposition occurs at all available sites. Thus, a first pattern may be formed, and the regions thus specified caused to react with a first chemical compound by contacting said surface with said first chemical compound or solutions thereof, a second pattern

may be formed and the regions thus specified caused to react with a second chemical compound by contacting said surface with said second chemical compound or solutions thereof, and so on, until patterns of all desired patterns of all desired chemical compounds (comprising the corresponding distinct affinity moieties) are formed.

After the polymerization of such a patterned layer into a sheet-like polymer, it is released from the initial patterned surface, e.g. by exposure to elevated temperatures. The affinity groups on each side of said sheet-like polymer may then be used, by similar steps as those used to produce said sheet-like polymer, as a template for the formation of complementarily patterned sheet-like polymers by exposure to appropriate affinity group terminally functionalized layer forming monomers. Repetition of such layer forming steps thus provides geometric amplification (here for fabrication purposes) of the patterned sheet-like polymer, in analogy to PCR, provided that the sheet-like polymer produced at each step is freed, as above, from the template surface which directed its formation.

Patterns thus generated may be designed to facilitate orderly binding together of such layers under appropriate conditions (e.g. thermal annealing) into bi-layer or multilayer structures, in analogy to the hybridization of DNA molecules. Such design will favorably include pattern elements that ensure proper sheet-like alignment with proper registry before bulk or random associations between surface regions of said layers are permitted to occur.

For example, considering a square sheet-like structure, at sufficiently low concentration to preclude significant trimolecular or tri-complex reactions, one corner of each of two sheet-like structures to be juxtaposed may comprise thiol groups, which thus target these corners to each other before affinity interactions are permitted to occur. A second set of corners, diagonally located from said first set, may comprise primary hydroxyls, which are then crosslinked by an appropriate crosslinking agent, such as a bifunctional acid anhydride compound. Thus, a first set of corners are targeted together, followed by the diagonally opposite set, after which registry of appropriately designed affinity patterns causes registry of the remainder of the juxtaposed surfaces.

Where sets of affinity group terminally functionalized layer forming monomers in which different affinity groups correspond to

different intermediate (intervening) structural member composition, and hence physical properties, layered and multilayered

Rapid Formation of Precise Thickness Multilayers:

Where multilayers comprising many molecular layers with monolayer precision are required, methods more rapid than sequential monolayer deposition are desirable. This may be accomplished by forming a Langmuir-Blodget film on a solid surface which may later be dissolved by a solution which will not disrupt or destabilize the overlying layer. This surface is again used to lift an interfacial layer, such that there are now two layers situated on said surface. Dissolving such a substrate permits the overlying layer to be situated at a phase boundary, and is favorably accomplished by slowly lowering said substrate into said solution which dissolves it, such that once it is dissolved, said overlying layer is situated at said phase boundary. After the preceding steps, there would be two stacked monolayers at this point. Conditions (e.g. temperature, composition, etc.,) are adjusted such that a dissoluble solid may again be passed through said solution without dissolution, to capture said overlying layer previously situated at said phase boundary. Said surface with said overlying layers is again used repeatedly to capture layers of a precise thickness, which thickness is determined by the preceding liberation step. The number of capture steps and the layer thickness thus determine the precise number of monolayers added before a substrate is dissolved, liberating the resulting multilayer at said phase boundary. Thus thickness may increase multiplicatively rather than in increments of a single monolayer or a single bilayer. Thus, such a method comprises the steps of: forming an interfacial layer, e.g. from amphiphilic molecules at an air water interface; depositing such a layer on the flat surface, preferably of a dissoluble solid; repeating this deposition step at least one more time; liberating the multilayer thus produced from said surface, preferably such that it is situated at a phase boundary and preferably by dissolving said solid in a solution which will not disturb the structure of said multilayer; and, repeating this process one or more times with said multilayer, such that such repetition yields a multiplication in the thickness of the preceding multilayer.

The principal limitation to this method is that the sum of the area of the monolayers of the resultant multilayer will be conserved with respect to the area of the initially formed monolayers or bilayers.

Means other than ordinary dissolution may also be used to liberate multilayers. For example, a substrate used with this embodiment may be of a polymeric composition comprising photolabile linkages. Said polymeric composition comprising photolabile linkages is preferably chosen such that the photodegradation products will be soluble in the solution used to form interfacial films or to support thus liberated films, and to not bind with or otherwise interfere with these films. Subject to these limitations, a wide range of compositions are suitable for substrates. Where substrates are of polymeric composition, these are most preferably cast with a highly flat surface, simple examples of which include glass and freshly cleaved mica. After each addition of layers as described above, substrates are floated or otherwise held at the surface of the solution to support the overlying film supported by said substrate after dissolution of said substrate, and the substrate is exposed to a sufficient intensity of light of a frequency chosen to cause the degradation of said photolabile linkages for a sufficient length of time to effect complete dissolution of said substrate, with further time being allowed as necessary for degradation products to diffuse away in this solution from the interfacial layer.

Note that such a process may alternatively avail a release layer, which may be produced as a self-assembling monolayer, for example comprising photolabile or thermolabile linkages situated on the surface of an insoluble substrate. Here, said substrate and release layer are lowered into a solution which will support the overlying layer or film as a physical treatment disrupting said release layer is effected such that said overlying layer or film is lifted by said solution from said substrate, the bonds comprised in the structure of said release layer having been disrupted. The composition of said release layer is preferably chosen so that the disrupted layer subsequently produced is well wetted by the solutions used, and such that the free degradation products (those not bound to the substrate surface) are well solvated by the solutions used.

Additional Embodiments:

Molecular and Supramolecular Synthesis with Positional Control:

This embodiment describes first devices for the parallel positional synthesis of molecular and supramolecular objects or articles (termed workpieces or workpiece precursors) which may also be devices, methods for the fabrication of said first devices according to the general microfabrication methods of the present invention, and methods for the use of said first devices to effect positionally controlled molecular and supramolecular synthesis. Terminology used will include terminology from the field of art of scanning probe microscopy.

Surfaces arrays of Z-actuators, each under independent control are produced, for example, by the foregoing methods of the present invention according to the following design. A capacitance based actuator is produced by forming electrodes on a surface which serves as a first plate of a parallel plate capacitor. Two categories of capacitive actuators are possible: unfilled (i.e. air or vacuum gap) capacitors where the coulombic force between capacitor plates arising from the charge separation due to applied voltage difference is opposed by forces associated with mechanical flexure of a second capacitor plate partially or completely suspended over said first plate such that a cantilever arrangement results; and filled capacitors wherein said first plate is coated with an elastiomeric or other compressible substance, according to the molding or casting methods of the present invention, upon which a second plate of a parallel plate capacitor is formed, where compression of said elastiomeric or other compressible substance opposes the coulombic forces resulting from charge separation across the gap of said parallel plate capacitor. Note that for filled capacitors, composition of the substance between capacitor plates may further be chosen according to dielectric properties in addition to mechanical properties. The functional characteristics of both categories of actuators will be highly dependent on geometrical factors and on materials properties, providing a large range of realizable device characteristics according to tradeoffs elected in design and fabrication. Each such capacitive actuator may be placed under control of a different voltage signal, under electronic control, favorably with the electronic circuitry effecting this control integrated into the same array device, according to conventional

electronic device integration design practices and, for example, the microfabrication methods of the present invention, such that each capacitive actuator may be translated to a position independently of other capacitive actuators in the same array. For purposes of the present description, the direction normal or perpendicular to the surface upon which said array of actuators is formed shall be denoted as the Z-axis, which is also the axis substantially perpendicular to the plane of the plates of said parallel plate capacitor actuators. An integrated array of Z-actuators is thus produced on a substrate surface.

The portions of said Z-actuators furthest from the substrate (i.e. the topmost capacitor plate) may further comprise or be in communication with a small cantilever, the purpose of which is to facilitate the measurement of forces acting upon any tips associated with said cantilever. Said forces may conveniently be measured by reflecting a laser beam from the bottom surface (i.e. the surface nearer to said substrate, which in this instance is chosen to be transparent) and monitoring and changes in the reflected angle of said laser beam (e.g. with a split photodiode arrangement as is commonly used as detection means in AFMs), or by other means which have been employed in the design of AFM cantilever deflection monitoring means (e.g. capacitive detection, tunneling detection, piezoresistivity change detection, etc.,.)

In the case of tunneling detection, a cantilever is coated with a conductive layer on the bottom surface of said cantilever, which is juxtaposed with a tunneling contact. The extent (proximity) of said tunneling contact to said conductive layer on said cantilever may be self-aligned by electroplating at said tunneling contact until a desired current is observed between said conductive layer and said tunneling contact, while said cantilever is at its equilibrium (undeflected) position, optionally followed by a brief reversed potential to dissolve a very small portion of the deposited metal from the surface of said tunneling contact to establish a predetermined gap size. Detection methods relying on such structures may additionally rely on detection of variation in field emitted current between said conductive layer and the conductive structure termed a tunneling contact above in the case of tunneling current based deflection detection.

The signals corresponding to the deflections thus detected may, as in conventional AFM systems, be used to adjust the position of said Z-actuators to restore a desired set point value. As will be apparent to those skilled in the arts of SPM and SPM design, other regimes of AFM and SPM will be possible with the appropriate modifications or extensions of the present array format.

The externally exposed surface of the topmost plate of said parallel plate capacitor, or layers formed upon said externally exposed surface may be further patterned such that a confined surface region, of predetermined size and surface elemental or chemical composition is produced in communication with the moving portion of said capacitor actuator. In the simplest case, a masking pattern may be formed on the exposed surface of a gold said topmost plate such that only a small region, for example smaller than 100nm by 100nm square of bare gold remains exposed. This surface thus comprises a localized region or pad in and X-Y array of said actuators, which may be translated in the Z direction independently of other similar localized regions or pads of other actuator array elements.

Patterned Molecular Tip Arrays:

In one variant of this embodiment, said square of bare gold may be used as a target for the random adsorption of organothiol molecules or molecular complexes. Such adsorption is preferably carried out such that deposition of said organothiol molecules or molecular complexes occurs at a rate of one per pad or region, i.e. preferably exactly one per actuator, but at least on average one per actuator in the case of random deposition. In other variations, small objects may be substituted for said organothiol molecules or molecular complexes, in which case the said externally exposed surface or coatings thereupon are chosen so as to stably bind the corresponding said small objects selected. Said small objects may be selected from among: macromolecules, enzymes and conjugates thereof, biological receptors, immunoglobulins or fragments thereof or conjugates thereof, colloids, nanocrystallites, polymeric beads, dendrimers, fullerene derivatives, nanotube derivatives, mesoscopically structured single objects or other small objects of predetermined geometries or geometrical families. These molecules, molecular complexes or other small objects serve as an extremities or projections in communication with actuator

pads; these will be generically referred to herein as tips. Note that other types of tips include any of the above small objects in communication via a flexible molecular linker to a chemical functional group which binds stably to said pads, such that said small object is positionally constrained according to the flexibility and tethering of said linker, but not precisely positioned. Further, another type of tip may be formed by electron beam deposition of vaporized substances onto said pads.

Note that actuator arrays may homogeneously consist of a single pad type decorated by a single type of tip, multiple pad types (i.e. multiple pads of each type where each of said types has a different surface chemical composition) decorated on a one-to-one composition basis by corresponding multiple small object types, or may comprise different pads decorated, according to foregoing spatially predetermined copolymer synthesis methods, with distinct oligonucleotides, polypeptides or other copolymers with distinct binding properties.

Arrays of workpiece pads are produced by forming small exposed regions of a surface of a convenient composition on a substrate surface, such that said workpiece pads may line up with actuator pads and/or tips of the above actuator arrays. Workpiece pads may also comprise or overlie actuators, which may for example serve to tilt said workpiece pads relative to said tips, or may merely comprise pad arrays which facilitate the colocalization of tip and workpiece, depending on the design of the article of fabrication and the tip or tips used.

In particular, molecular tips within tip arrays (tip flats) such as those described by Drexler[53] may be selected according to the tilt of said workpiece pad effected by one or more actuators which adjust the angle of the plane of said workpiece pad relative to the normal defined by the Z-actuator of the tip pad. Note that the tip arrays described by Drexler pertain to conventional atomic force microscopes and cantilevers used therewith, said cantilevers modified to further comprise said tip flats. To perform the corresponding synthetic operations in parallel, rather than on a single workpiece, the present invention provides that an array of actuators positions molecular tips or arrays thereof along the Z-axis, while one or more actuators

associated with each workpiece pad orients said workpiece pad to orient said workpiece pad such that one molecular tip of the opposed array is selected according to the orientation dependent proximity. Such molecular tip arrays may, following Drexler, be situated on a curved surface, here each in communication with one of said Z-actuators, where the approach of a particular molecular tip in one of said arrays is selected according to the tilt of the workpiece relative to said one of said arrays which selects a distinct normal to said curved surface, thus selecting said molecular tip situated on said curved surface at the locus of the line normal to both said curved surface and said workpiece pad (i.e. the point of contact as said curved surface would approach said workpiece pad, assuming convex shape.) Thus, provision of workpiece pads comprising one or more actuators effecting tilted orientation of said workpiece pads relative to the Z-axis translation of said Z-actuators of said tip pad arrays permits the use of arrays of tip arrays in the parallel modification of arrays of workpieces. If such tilt is not effected by arrays of actuators, only one workpiece may be produced at a time benefiting from the selection of particular individual tips (one at a time) from among the multiple tips in a tip array, because the appropriate workpiece substrate alignment for one workpiece will preclude interaction of other workpieces on the workpiece pad array from being juxtaposed with the pads of said tip pad array. The particular advantage of being able to select individual tips from an array of tips in the modification of a workpiece accrues from the ability thereby to selectively interact said workpiece with a different, predetermined chemical functionality or composition of said individual tips, while maintaining a deterministic relationship between the spatial location of said tip array with said workpiece (i.e. eliminating the need to locate a workpiece upon changeover of tips.) An array of tilt actuators permits such advantage to be availed in combination with the advantages of parallelism accruing from the simultaneous positionally controlled modification of workpieces situated upon said workpiece pad array comprising said array of tilt actuators.

Local Vertical Positioning and Global Lateral Scanning of Probe Arrays:

Where conventional scanning probe microscope designs, including microfabricated implementations thereof,[54,55] comprise tips each having X, Y and Z actuators, greater ease of fabrication and greater X-Y positioning accuracy may be achieved in array format by producing an array of tips each situated on a distinct, independently controlled and monitored Z-actuator, where said array of tips overlying such an array of Z-actuators is translated by a single X-Y positioning means. While such an arrangement precludes independent X-Y motions of the individual tips of said array of tips, the elimination of separate X-Y actuators for each tip of said array of tips permits a higher areal density of tips (or tip pads in the terminology adopted above, as distinct from the molecular tips of a molecular tip array, which would be situated on a single tip pad of said tip pad array) than would be possible where redundancy of X-Y actuators occurs. Retention of independent Z-actuators in such a design permits maximally precise alignment of different tips with the corresponding opposed workpiece pads without variations in interaction forces between tips and workpieces which would result when cantilever flexion compensates for Z positional variation of said workpieces relative to said tips.

Where said X-Y positioning means is a piezoelectric tube scanner, additional care must be taken in calibration and scanning voltage waveform generation to ensure that deviations from planar orientation of the tube opening do not cause a rocking motion of the array mounted thereupon (i.e. compensation in the Z-axis at various points around the height of the tube scanner as a function of X and Y position). Less conventional piezoelectric bimorph actuators or capacitor actuators are thus favored in this regard, though other measures may ensure proper operation with piezoelectric tube scanners.

Self Alignment of Molecular Tip with Molecular Workpiece:

Where molecular tips or other molecular positioning members are randomly situated on said tip pads of said tip pad arrays, alignment with the molecular precursors of workpieces or workpieces situated on the workpiece pads of said workpiece pad array must be accomplished. This is most favorably accomplished by initially positioning said molecular precursors of workpieces on said workpiece pad array with said molecular tips or other molecular positioning members on said tip

pad arrays. Placement of said molecular precursors of workpieces or said workpieces on said workpiece pads of said workpiece pad array by said molecular tips or other molecular positioning members ensures correct relative positioning of said workpiece precursors or workpieces relative to said molecular tips or other molecular positioning members and the reactants later carried thereupon. Thus, random variations in the precise positions of said molecular tips or other molecular positioning members on the pads of said tip pad arrays pose no difficulty in alignment because such placement causes corresponding variations in the precise positions of said workpiece precursors or workpieces according to those of said molecular tips or other molecular positioning members, i.e. positions of molecules or complexes thereof on arrays are replicated, and such replication measures ensure good positional match between molecular tips or molecular positioning members and workpieces.

As an example, a molecular workpiece precursor, comprising a thiol group, may be deposited onto a workpiece pad comprising an exposed gold surface by a molecular tip, such that the resulting position of association of said thiol with said gold surface corresponds to the position on said tip pad or cantilever of said molecular tip. In a further extension of this example, said workpiece precursor may additionally comprise a cleavable linkage susceptible to a particular predetermined physical or chemical treatment, and a first reactive group, for example a hydroxyl. A second molecular workpiece precursor molecule, for example of identical composition, may be deposited by the same process at a second location on said workpiece pad. Thus, two predetermined reactive groups are placed in a predetermined arrangement on said workpiece pad. As desired, a third molecular precursor molecule may similarly be positioned at a third point, in which case the three positioned reactive groups thus fix a coordinate system admitting no gross rotations (i.e. further exclude the rocking motions possible in structures anchored with only two reactive groups fixed on a surface.) Following the placement of such workpiece precursors, molecular components may be reacted with the reactive groups of said workpiece precursors according to the methods described below.

Note that further self-alignment may be achieved at an earlier step by replicating the flat surface of the substrate to be used to produce

said workpiece pad array from that to be used to produced said tip pad array. Such a step will ensure that local variations from planarity (e.g. curvature) in the surface of said tip pad array are compensated by the corresponding variations thus produced in the replicated workpiece pad array substrate surface. The necessity of such steps in practice will depend on the size of such surfaces and the ability to obtain or fabricate ideally flat substrate surfaces as materials for the production of tip pad arrays and workpiece pad arrays.

Constraint-Based Simplification of Positional Synthesis:

A more immediately practical approach to the positional synthesis proposed by Drexler fabricates structures from structures larger than single atoms, and avails conventional solution and solid-phase chemistry where positional control is not essential. These methods permit the synthetic fabrication of extended, complex heterogeneous structures which would be difficult or impossible to produce by known art chemical methods.

Most conveniently, the size of molecular components or reactants and the linkers which hold these in communication with said molecular tips or molecular positioning means are chosen to be of a sufficiently great size that angstrom positioning accuracy is not strictly required to exert positional control over synthesis. Instead, said linkers, which may for example be composed of a polymethylene (polyethylene) polymer chain or other preferably straight chain flexible polymer chain, which are attached via some cleavable linkage to said molecular components or reactants, constrains said molecular components or reactions to a volume defined by the linear length of said linkers (and the size of said molecular components or reactants) with a probability distribution (or effective concentration as a function of position within said volume) which may be predicted using Flory theory. Said volume to which said molecular components or reactant may be termed a constraint volume. Positional control over synthetic reactions involving said molecular components or reactants and said workpieces or workpiece precursors inheres in the X-Y translation of the substrate comprising said tip pad array and the Z-translation of said tip pads, such that the resultant three dimensional positional control translates said constraint volume which contains said

molecular components or reactants (more specifically, the reactive chemical functional groups thereupon) into proximity with the desired reactive sites on said workpiece. In other words, control over translation of said constraint volumes is used to select which reactive sites of said workpieces are reacted. Thus, the resolution of such a method is limited by the extent of said constraint volume, and workpieces are preferably designed according to said resolution (otherwise resultant workpieces will obey the corresponding probability distribution related to the number of possible sites within an approached constraint volume and the effective concentration distribution at each of said possible sites.)

Note that such a linker may alternatively have a large central portion of their length composed of a rigid polymeric member (with terminal flexible polymeric linkages), in which case the attached molecular components or reactants are confined rather to a shell, which may be termed a constraint shell. The configuration of said constraint shell is determined by the structure of the partially rigid said linker, i.e. defining a volume beyond which the terminally situated said molecular components or reactants may not extend and also a closest point of approach to the point on said tip pad at which said partially rigid said linker is attached beyond which said molecular components or reactants may not approach. In this case, the effective concentration of said molecular components or reactants within such constraint shells are correspondingly higher as compared with the above described constraint volume corresponding to a completely flexible structure. Note, however, that greater positioning accuracy may be required in these instances, but such accuracy should be well within that practically achievable with current instrumentation.

After the positionally constrained reactions occur according to the above described steps, communication of said linkers and said workpieces, which results from any bonds formed by said reactions, may be tested. This may be accomplished by monitoring any forces exerted by said communication as said tip pad array is withdrawn from said workpiece pad array or, alternatively, as said Z-actuators of said tip pads are translated away from said workpieces. Said forces may be monitored conveniently if each of said molecular tip (or said linker) is in communication with a cantilever, the position of which is

tracked by any known art method such as optical beam deflection, monitoring of a tunneling current between said cantilever (coated by a metal) and a tunneling tip, changes in piezoresistivity, changes is capacitance where an electrode situated on said cantilever forms one plate of a capacitor, etc.,.

Two possible cases occur. First, communication of said tip with said workpiece may be detectable as cantilever deflection upon retraction of said tip from said workpiece, while said retraction does not sever said linkage. Alternatively, said retraction may sever said linkage. These two cases will have different requirements and advantages.

In the first case, a linker is preferably of elastiomeric composition such that tension is gradually applied to said workpiece as deflection of said cantilever occurs during said withdrawal. In this way, gross severing of linkages, which would be expected with applied tensions of less than 10 nN for single bonded linkages, are avoided. Otherwise care must be taken to ensure that applied tensions remain under a tolerable maximum to prevent such severing. In such cases, once a reaction between said molecular component and said workpiece has occurred, the tip remains in communication with said workpiece, until chemical or physical treatment severs the resulting linkage. Linkages (i.e. linkage composition) is chosen according to the desired methods of linker cleavage. For example, linkers may comprise bonds susceptible to cleavage by particular chemical reagents, may be heat labile or may be photocleavable, e.g. comprising esters to compounds such as nitroveratryloxycarbonyl compounds, such that said ester linkages are cleaved by exposure to appropriate wavelength actinic radiation.

In the second case, such as the case for molecular components bound to said molecular tip or molecular positioning means via affinity interactions consisting of weak forces (e.g. hydrogen bonds, van der Waals forces, solvation energies) withdrawal will lead to severing with a predetermined applied force, which will be detected as the corresponding cantilever deflection. In the case of oligonucleotide based affinity interactions, the observations of G.U. Lee, L.A. Chrisey and R.J. Colton[56] are indicative of the kind of affinity interaction severing forces expected. This second case, with oligonucleotide binding, is preferable in that well characterized

binding interactions may be used to effect rapid, reversible binding of molecular components to molecular tips or other molecular targets (i.e. molecular positioning moieties) comprising the complementary oligonucleotide sequence.

In either case, reactant molecular components are situated on said molecular tips or tip pads by contacting said tip pad array with a solution comprising the said reactant molecular components to be added to said workpieces during the subsequent reaction step and permitted to bind to the available sites on said tip pad array, i.e. to molecular tips or to the affinity groups thereupon.

Once a molecular component has reacted with the specified site on a workpiece or workpiece precursor, and has been severed from the molecular tip or molecular positioning means, if any workpieces on said array of workpiece pads did not evidence communication with said cantilever (i.e. negative deflection upon tip withdrawal), and yield is to be maximized at the expense of the repetition of steps, the preceding steps from binding of molecular components to molecular tips or molecular positioning means on said tip pad array through said withdrawal step may be repeated with the same molecular component and positioning. Sites which had previously been reacted will lack active reactive sites, while those which did not successfully react will experience a repeated opportunity to react with the desired species. After an arbitrary number of failures, a workpiece may be deemed to be mispositioned or otherwise refractive to correct assembly.

Constraint based simplification applies not only to constraint of reactants (i.e. molecular components) to tips, but also applies to the design of molecular components themselves and the design of the workpieces which they are used to assemble. A molecular component will generally be bound to a workpiece at more than one location, i.e. will generally have greater than one point of connectivity with said workpiece. This is most simply and rapidly accomplished with only one positioning step per molecular component, with the formation of subsequent linkages constrained by the geometries of said molecular component and the structure, at that step, of the workpiece. In other words, a positionally controlled reaction links such a reactant molecular component to a first reactive site on a workpiece, and the structure of said reactant molecular component and said workpiece are

such that the reactive groups on said reactant molecular component and said workpiece will react together for only one relative topological orientation of said reactant molecular component and said workpiece. For rigid structures (i.e. those comprising rigid polymeric structures) simple design rules may yield such a result, namely, that the pairwise distances of all similar reactive groups are different and that once a first bond is formed, under positional constraint imposed by linkage to said tip, no incorrect second bonds to reactive groups on said workpiece may form after release of said reactant molecular component from said tip. In other words, no rotation of said reactant molecular component about the first bond formed under positional constraint by said tip will yield proximity of reactive groups situated on said reactant molecular component to correspondingly reactive groups of said workpiece except that rotation which permits the desired reaction and hence the desired connectivity.

Note that flexible components may also be employed according to such constraint-based design methods, but here flexibility entails that the positional constraints on reactions of these components obtaining after a first bond or linkage is formed between such flexible components and a workpiece will concern the maximum possible reach of other reactive groups on said flexible components. Thus, the selection of rigid or flexible components for different parts of a workpiece structure will be according to considerations concerning both the desired structure and the preferred molecular assemble strategy. Note, however, that with both flexible and rigid components, and also with components comprising both flexible and rigid regions, component sets may be synthesized comprising different topologies such as different degrees of branching, different lengths of branch arms, different chemical functionalization of regions or location of said branch arms, etc.,.

Aside from the above described positionally constrained reactions, workpieces may be treated with other reactants or chemical modifying reagents from bulk solution. For example, reactive groups on molecular components may all be primary hydroxyl groups. Once said molecular components are incorporated into workpieces (and released from said tips), and said tip array withdrawn from said workpiece pad array, said hydroxyls may be modified to be reactive to other hydroxyls, e.g. by reaction with bivalent cross-linking agents

(provided in excess) comprising two acid anhydride or two acid chloride functionalities. The first such functional group of each such cross-linking agent molecule will react with each hydroxyl on said workpiece, while the second such functional groups of each such cross-linking agent molecule, now a reactive group on said workpiece, will only react with approaching hydroxyls of new molecular components. Of course, this entails that no two hydroxyls available on a workpiece may be closer together than the length spanned by the reactive functionalities of such cross-linking groups. Alternatively, molecular components may carry protected functional groups, which, once said molecular components are reacted onto said workpieces and released from said tips, are deprotected by contacting said workpiece pad array, as in the case of activation with cross-linking agents, in this case with deprotection reagents. In either case, function groups which were held inert with respect to each other to prevent unwanted reactions between molecular components as these are prepared and bound to said tips, are activated once on said workpiece. In the case of acid chloride or acid anhydride chemistries, inert solvent media must be chosen. Further, control over the steps at which functional groups on said workpieces are activated or deprotected provides another method of control over synthesis, i.e. a method by which a subset of functional groups are held inert until a desired assembly step, whereupon they are deprotected or activated by treatment with appropriate reagents. It will be obvious to those skilled in organic synthesis that other reactive chemistries, as will as multiple, concurrently used chemistries will be possible and often preferably advantageous over the simple example presented here merely for purposes of illustration.

Note that molecular tips and any affinity groups thereupon may thus be spared from exposure to highly reactive groups (e.g. oligonucleotide moieties of molecular tips need never be exposed to acid chloride containing compounds, which would inactivate said oligonucleotides for proper binding).

As an example, reactive groups may be primary hydroxyls on alkyl chain linkers, rigid structural members may comprise polyparaphenylene derivatives[57] or the phenylacetylene polymers disclosed by J. Zhang, J.S. Moore et al.[58], in either case comprising components with structures synthetically accessible, for example, by the methods of J.

Zhang, J.S. Moore et al.,.[59] Affinity members for reversibly binding these components to molecular tips may comprise oligonucleotides, oligopeptides, peptide nucleic acid oligomers or small molecule haptens joined to said components (e.g. via methylene linkers), according to the complementary affinity members on said molecular tips. Other molecular component structures of interest in the construction of molecular devices by such assembly methods include rotaxanes comprising macrocycles which themselves comprise reactive groups, and catenanes. Molecular components based on these topological compounds provide structures useful as moving parts in objects and devices fabricated by these synthesis methods.

Affinity groups such as oligonucleotides may be included within the structures of said molecular components or may be positioned by tips into proximity with proximity selected reactive groups on said workpieces which will react with linkers (e.g. at terminal hydroxyls) attached to said affinity groups. Note that if workpieces are to subsequently be exposed to reactive compounds, said affinity groups may be provided with any potentially reactive functional groups protected by protecting groups, which may be removed in a final step after such exposure. Note that such affinity groups to be included in the final structure of said workpiece must thus be protected to ensure that these are not derivatized in undesired ways by synthesis reagents, but affinity groups used to bind reactant molecular components to said molecular tips or molecular positioning means may be sacrificed. Said affinity groups used to bind reactant molecular components to said molecular tips or molecular positioning means may be in communication with the remainder of the structure of the reactant molecular component which they serve to target to said molecular tips or molecular positioning means via linkers cleavable by predetermined physical and/or chemical treatments (e.g. hydrolysis of an ester linkage) such that these moieties may be removed from said workpiece after they are no longer needed. Of course, such removal must be accomplished according to chemistries which do not adversely affect the remainder of the workpiece structure, and are thus to be chosen according to such design considerations.

Note also that similar positional constraint methods may be used to effect positionally controlled deprotection of reactive groups, such

that the position of a tip is used to select which reactive group or groups on a workpiece, out of several groups of similar composition at different locations on said workpiece, will be active during a subsequent reaction step where the reactant molecular components react with such deprotected or activated chemical functional groups on said workpieces directly from solution. This variation adheres to the same basic principle of selective positional modification of chemical functional groups, here preparing their activity for subsequent synthetic steps. For example, chemical functional groups may be primary amines, which may be protected by esterification. Here, a molecular tip may comprise or may be linked (e.g. by known art conjugate chemistry) to a protease enzyme molecule, such as Proteinase K. Only those primary amines within the reach of such enzyme molecules tethered to a molecular tip or tip pad are susceptible to enzymatic deprotection, and hence only the subset of such amines on said workpieces will be deprotected. Other catalysts such as ribozymes, metal tip surfaces, or metallic colloids partially embedded in polymer matrices may similarly be used, as may tethered molecules which effect deprotection by non-catalytic reactions.

Nanoscale Data Pattern Replication and Readout Methods and Means:

The large data capacities realizable with the various data storage methods comprising the use of scanning probe technology based data readout are not accompanied by a commensurate increase in the rate of data recording except where large numbers of scanned probes are used to record a data pattern onto a storage medium surface. Because one particular advantage of such storage technologies is in applications related to data publishing, a rapid method for the duplication of predefined bit patters readable with such readout technologies would be desirable, as has been the case with read-only optical data storage technologies.

The methods of the present invention are well suited to the fabrication of surfaces with inscribed nanoscale data patterns readable with various scanning probe readout means. These include near-field photon transfer (corresponding to NFSOM), electron tunneling and electron field emission (corresponding to STM and field

emission mode STM), and surface profile detection (corresponding to AFM and variations thereon).

In the case of photon transfer, a transparent surface such as glass or polycarbonate is coated with a metal film, which is patterned by the foregoing methods of the present invention for the spatially predetermined protection or masking of metallic film surfaces from etchants such that the desired bit pattern is formed comprising apertures in said metallic film and intact regions in said metallic film which reduce or eliminate the local transmission of light. Such a bit pattern may be read out by conventional NFSOM apparatus or variations thereupon, or the near-field detection means described below. The patterned said metal layer may mask the local transmission of photons to a probe aperture which conducts received photons to a detector, said photons originating from a source on the opposite side of the transparent material comprising said transparent surface, in which case said metal layer serves as a photon transmission mask. Alternatively, said pattern in said metal layer may mask the transmission of photons emitted due to the fluorescence of compounds in said transparent material excited by photons either emanating from an NFSOM tip aperture which may be the same aperture used for the detection of fluorescently emitted photons or emanating from a source on the other side of said transparent surface.

Similarly, in the case of electron tunneling transfer or electron field emission transfer, a metallization pattern may similarly be formed, here on an insulator, or alternatively a relief pattern may be formed in one or more metal layers with all exposed surfaces having conductive properties. In either case, a bit pattern which may be replicated by the methods of the present invention is encoded in such a surface relief pattern. Such a bit pattern may be read out with instruments such as STMs, microfabricated STMs or the array devices disclosed herein comprising plural Z-actuators each associated with a tip, which entire array is scanned across a juxtaposed surface by an X-Y positioning means and wherein data patterns are detected by monitoring, with electronic means, voltage, current or height of each tip either singly or in the various possible combinations and where said current may be either a tunneling current or a field emission current according to particular implementations.

In the case of AFM or other surface height responsive data readout means, relief surfaces comprising surface patterns are replicated by casting, injection molding or embossing methods.

In all of these cases, an original relief comprising the desired bit patterns may be formed by scanning probe technology based patterning methods such as those of related art reviewed above.

By such methods, large numbers of replicas of an initial surface pattern encoding information may be rapidly and inexpensively produced, permitting economical data publication in high density formats readable by scanning probe based data readout technologies.

Simplified Method and Means for Data Readout by Near-Field Optical Scanning:

A CCD array, fabricated by methods of the present invention or by conventional methods may be modified to serve as a highly parallel detection means for optical data readout relying on near-field photon transmission through a small aperture. An array of waveguide elements, for example composed of PMMA, is molded onto the surface of said CCD array such that each photodiode of said CCD array will capture photons conducted by each of said waveguide elements. The array comprising said waveguide elements must therefore be aligned with the photodiode array of said CCD. Gaps between said waveguides are filled in with, for example, an opaque polymeric material, or may be electroplated provided this will not cause short circuits in said CCD, which may be designed to prevent such problems. Said waveguide element array and any material filling said gaps are formed such that a substantially flat surface results. A metal film, preferably of gold, of sufficient thickness to prevent appreciable transmission of photons is then deposited onto said surface. Said film is then masked such that only one small pinhole will be etched overlying each photodiode and associated waveguide; said pinhole will serve as an aperture limiting photon tunneling. This may be accomplished by patterning with organothiol compounds using an elastiomer, according to the method of Whitesides and co-workers, followed by an acid etch step, by the related multilayer vetoing methods described herein, or by the other patterning methods disclosed herein. Thus, an array of pinholes of predetermined size and location is fabricated with associated waveguides, which conduct any photons received at the

aperture formed by said pinholes to the photodiodes of said CCD in a one-to-one manner.

Such an array of pinholes with associated waveguides may also be fabricated by LIGA methods and variations thereupon, and then situated in an appropriately aligned manner onto a CCD array surface. Alternatively, the same type of structure comprising an array of pinholes with associated waveguides may be fabricated by other molding and electroforming methods described herein and then, similarly, situated in an aligned manner onto a CCD array. Such an array may be termed an aperture limited CCD array (ALCCD).

The CCD array devices used in this aspect of the present invention are preferably of high sensitivity, such that a reduced number of photons must be received by each photodiode to register a signal.

Such an ALCCD may be used for data readout by juxtaposing the surface of said ALCCD comprising said pinhole array to a patterned metal film surface of a transparent material such glass, such as that described above which pattern may be replicated as described above. Said pattern metal film surface is held parallel to the surface of said ALCCD comprising said pinhole array, at a distance of less than one half the wavelength of the light employed and preferably at a distance of less than one-quarter of this wavelength. Smaller separations are further preferred. The region of said patterned metal film opposite each pinhole on the surface of said ALCCD is either be coated with said metal film or may expose the underlying said transparent material, which distinction provides for the detection of the corresponding data pattern. Thus, the density achievable with this data readout apparatus and method is primarily determined by the dimensions of said pinhole, which limits the photons thus received to those originating from narrow exposed regions.

Said patterned metal film surface is scanned, in a manner maintaining parallel alignment with the pinhole array surface of said ALCCD, such that an area corresponding to the size of each CCD array element passes opposite each pinhole, by repeatable positioning means such as piezoelectric or capacitor actuators. In other words, said patterned metal film surface is scanned in the X and Y while maintaining a substantially constant Z separation, such that the signal received by each photodiode of each CCD element correlates, according to scan timing, with a particular position on said patterned

metal film surface. Scanning is conducted slowly, such that said CCD captures the bit image corresponding to a particular position of said patterned metal film surface, which is then read out from said CCD according to conventional methods associated with the use of such electronic imaging or memory devices. Thus data rates will primarily depend on the device characteristics of the CCD used. The X-Y location of said patterned metal film surface relative to said ALCCD are then translated by incremental scanning motion of said actuator, and the bit pattern associated with this subsequent position is then similarly collected.

In the simplest instance of this method, photons originate from a source on the opposite side of said patterned metal film than said ALCCD, with transmission occurring through exposed regions of said transparent surface as it is masked by said patterned metal film. The number of photons received by each CCD element are limited by the relative size of said pinhole to the area of each CCD element. Thus it is desirable that either high intensity sources are used to compensate for this limitation, or that incident light be focused so as to reach the pinholes situated on the surface of said ALCCD at a sufficient areal intensity. This may be accomplished by the use of a microlens array, for example such as that described by H.A. Biebuyck and G.M. Whitesides,[60] with appropriate focal length of the lens elements which it comprises, such that incident light is concentrated as it passes through said patterned metal film surface and reaches said pinholes on said ALCCD surface. Said microlens array is thus constructed at a similar density as the CCD which is used, and is aligned with the surface of said ALCCD such that said pinholes are each at the focal point of the corresponding lens. In this case, said patterned metal film surface is situated between said microlens array and said ALCCD, and remains in a fixed position relative to said ALCCD as said patterned metal film surface is scanned between said microlens array and said ALCCD surface.

Method and Means for the Ordered Dissection of Chromosomes, Chromatin and Polynucleotides:

A relief is formed by replication methods at the surface of an elastiomer polymer composition, comprising a pattern of parallel lines comprising narrow raised regions generally of less than 200nm in width

and wider sunken regions preferably of greater than 1 micron in width. The aspect ratio of said narrow raised regions may be reduced by sloping the surface of said wider sunken regions in proximity to said narrow raised regions. Said polymer composition is chosen such that the resulting molecular network comprises chemical functional groups which may be reacted with cross-linking reagents to attach molecules to the surface of said relief. An appropriate cross-linking agent comprising a central linker of predetermined length, preferably shorter than 50nm is coupled to a nuclease enzyme (such as DNase I or a type II restriction endonuclease, preferably recognizing a 4 base restriction site) according to known art conjugate chemistry. Said cross-linking agent is chosen so as to link said nuclease to said elastiomer polymer comprising said chemical functional groups. A small volume of a solution of said cross-linking agent conjugated to said nuclease is spread on a surface as a thin layer in the pattern of a single stripe, preferably of less than a few millimeters in width. Said relief is contacted with said stripe of said conjugate on said surface, oriented with said parallel lines perpendicular to said stripe, such that nuclease molecules are linked to the surfaces of said narrow raised regions along the region juxtaposed with said stripe. The solution used to form said strip may favorably contain a dye or pigment to facilitate identification of the enzyme modified region, or other marking methods may be used to accomplish such identification. Thus, a relief comprising parallel lines and trenches of predefined size and location is produced with a predefined region of raised features upon which nuclease molecules are situated.

Linear or linearized DNA molecules or chromatin are treated so as to be immobilized at specific regions along their length, preferably near to one distinct terminus, and optionally derivatized with beads at the opposite terminus. For example, these may be hybridized with an oligonucleotide comprising a first affinity moiety such as biotin moiety and one or more psoralen groups (where plural groups are preferred to favor the formation of covalent linkages with both strands of the DNA double helix). After hybridization with said oligonucleotide, said psoralen groups are caused to react with said DNA to form a covalent linkages. Streptavidin is bound to a glass surface in a narrow stripe (<1 micron in width) shorter in length than the width of said strip of said conjugate above. Said DNA terminally

linked to said biotin is then contacted with said narrow stripe of streptavidin and affinity binding is permitted to occur. Then, for example, the immobilized DNA sample is optionally then briefly subjected to degradation by a 5′ to 3′ exonuclease, followed by treatment with a polymerase and second affinity group modified nucleotide triphosphates to terminally decorate said linearized DNA molecules with said second affinity group. Where modification with said second affinity group is performed, said immobilized DNA sample is then contacted with a solution comprising microscopic or sub-micron diameter beads, which may be fluorescently labeled, surface modified with receptors (e.g. immunoglobulins, etc.) according to known art conjugate chemistry. Alignment of the immobilized DNA molecules is then performed by known art techniques.[61] Said sample is then subjected to mild fluid flow in the direction perpendicular to said narrow stripe of streptavidin, as said glass surface is withdrawn from the liquid (or the liquid otherwise passes away from said DNA sample with an interface traveling in a direction perpendicular to said narrow strip of streptavidin). The molecules of said DNA sample are thus aligned in the direction perpendicular to said narrow stripe of streptavidin, fully extended, and situated on said glass surface. The same method may be applied to chromatin (first order packing of DNA with histone nucleosomes) or with condensed metaphase chromosomes. Straightening of terminally immobilized molecules may alternatively be accomplished, where said molecules underwent the optional addition of terminal beads as above, by methods such as those of T.T. Perkins et al.[62] whereby beads situated on DNA molecules are manipulated using an optical trap (also known as laser tweezers), or, where said beads are of paramagnetic composition, by the application of an appropriately directed magnetic field of sufficient strength to straighten, but not so large as to disrupt, the sample molecular and supramolecular structure.

Once sample molecules are end-immobilized to a precise, predetermined region, oriented and extended by means such as those above, the relief prepared above with raised narrow lines modified with nuclease molecules is then contacted with said glass surface on which said sample molecules have been straightened and aligned, in an orientation such that the parallel lines of said relief are perpendicular to said sample molecules, where said contacting is

performed under slight pressure to ensure sealing of the channels thus temporarily formed. A gentle fluid flow is immediately established in said channels, such that once a molecular region is freed at both ends by cleavage by said nuclease molecules, it is carried down said channel away from said nuclease molecules, which might otherwise further degrade it. Said flow then carries the cleaved molecule fragments to collection volumes formed in said relief structures at the ends of said channels, which are arranged such that the location of the channel in which a fragment was cleaved may be determined or inferred from the location of said collection volume. Thus, the collection volumes forms a linearly ordered set, which set comprises an ordered arrangement of fragments of a well defined physical length and precise ordering with respect to their original location in the in-tact sample molecules.

Such a method may be repeated, as desired, with a slightly different offset of said relief with respect to said narrow stripe of streptavidin, such that different regions are occluded from the collected sample set, to the extent that retention under said narrow raised lines poses any difficulties.

Note that in all steps, care is taken to avoid shearing sample molecules. Note further that slight differences in the positioning of the ends of immobilized sample molecules entails that the ordering of said fragments in a collection volume is better described as by a positional distribution function with respect to the sequence of the original in-tact molecules from which said fragments derive. Nonetheless, this simple process yields ordered fragment populations useful for establishing physical maps of genetic material and producing ordered samples therefrom for purposes such as library cloning or subcloning or other analysis techniques, and particularly useful in genome sequencing projects.

Two alternative genetic material dissection methods availing micropatterned reliefs are also possible.

In the first alternative, a relief comprising parallel raised narrow lines and trenches, such as that described above, is formed. This relief is then contacted with a first surface on which are coated particles such as metallic or glass colloids or hard microcrystallites, which are coated with chemical species capable of crosslinking said particles to said relief. Thus, said particles are

bound to the raised narrow regions of said relief. Genetic material is immobilized on a second surface and aligned as above, and the relief of the present alternative is oriented as above with said parallel lines perpendicular to the direction of alignment of said genetic material on said second surface and contacted with said second surface under an applied normal force. Liquid is caused to flow through the channels formed by said trenches and said second surface as a result of the preceding contacting step. The relief is then translated in the direction of said parallel lines as said normal force is maintained and said liquid flow is maintained. Thus, the genetic material is abraded and therefore cut by said particles, and once cut, fragments are freed from communication with said second surface and said relief, such that said fragments are carried by said liquid flow down said channels to collection volumes. The cutting action availed in the present alternative of this embodiment is in direct analogy to the cutting of DNA molecules by an atomic force microscope tip. Said second surface may be hard, such as glass, may be elastiomeric; said second surface may comprise bonded particles similar or identical to those used on said raised regions of said relief, in which case the cutting action may be compared to a scissoring action.

In a second alternative, a relief composed of opaque materials, comprising parallel lines of narrow raised regions and trenches is used, as above, to define flow channels when contacted under normal force with a second surface, here of a sheet-like or flat material. In this alternative, said second surface is transparent to radiation of the appropriate frequency range but is coated (preferably on said second surface, or instead on the surface not contacted with said relief) with materials which mask said radiation. For example, said flat material may be quartz coated with metallic mask materials and said radiation may be X-radiation, or said flat material may be glass coated with any material opaque to visible or ultraviolet light, which is used for irradiation. In the case of X-irradiation, genetic material exposed will be cleave directly due to double stranded breaks. In the case of ultraviolet or visible light irradiation, said genetic material is first bound by photochemical reagents which will cleave DNA when activated by radiation exposure; where said masking material is on said surface proximal to said relief, radiation may be

transmitted to said reagents in the near field regime, permitting resolution smaller than the wavelength used and equal to the masking material feature resolution. The masking pattern used is designed such that when said relief is contacted to said second surface, the lines of narrow raised regions are juxtaposed directly to unmasked regions of said second surface. Thus, said irradiation causes breaks of said genetic material at the loci of said narrow raised regions, which are in contact with said second surface, such that the fragments thus produced will be freed within the channels formed by said second surface and said trenches and carried away, by a fluid flow applied in said channels, to a collection volume corresponding to the respective channel.

Replicon Chimera Library Contiguity Analysis:

Articles produced by the methods of the present invention may be used to facilitate the analysis of polynucleotide libraries such as plasmid libraries, bacteriophage libraries, cosmid libraries, yeast autonomous chromosome (YAC) based libraries and the like. In the field of gene discovery and genome mapping it is often convenient to work with samples, generally in DNA form, which are fragmented and incorporated into replicon molecules capable of being maintained, replicated, expressed in and purified from appropriate host organisms. The fragmentation of genetic material which occurs in the preparation of such libraries, however, introduces difficulty into establishing the order with which the fragments thus obtained occur within the original, unfragmented polynucleotide sample. Further, mapping of genetic material over millions of base-pairs yields information useful in the field of genetics independent of the use of polynucleotide libraries. Therefore, a method which both provides rapid and economical genome or chromosome mapping, and is capable of determining the ordering relationship between the replicon chimera comprising a library would be of great use. Current art methods for accomplishing such tasks generally rely on the probing of arrays or replicas of clonal replicon-chimera isolates, whether in the form of colony or plaque isolates or arrays of spots of genetic material from such a library, generally rely on the transcription of RNA from bacteriophage promoters at the borders of the replicon used to create the library and directed such that sample fragments are thus transcribed.

Generally, such transcripts are transcribed with radiolabeled ribonucleotides, and the RNA thus obtained is used to probe said arrays or replicas; those array elements or colonies or plaques which hybridize the radiolabeled RNA transcripts thus share common sequences with the fragments from which said transcripts were obtained. Thus, these methods determine which clonal isolates contain common sequences, and by repetition identify series of clonal isolates which are of contiguous origin. Because such libraries may contain well over 10^4 distinct chimera, such information is rarely if ever exhaustively determined for any given library, and is usually only applied to small fractions of such libraries.

Copolymer arrays such as those disclosed in related art or produced by the methods of the present invention may gainfully be applied to the task of determining the linkage relationship between the individual chimera of a library and also to chromosomal mapping thereby. In this method, each replicon must be rendered uniquely distinguishable, for example by the incorporation of a sufficiently long random nucleotide sequence (e.g. 9 variable base pairs [which need not all be contiguous] in a replicon designed for use in preparing libraries of which $<10^5$ clones will be used.) Such a sequence will be referred to as a tag sequence, but it will be understood by those skilled in the relevant arts that different tagging and discrimination methods (e.g. the use of epitope library fused terminal-protein replicated viral or bacteriophage [e.g. PRD1, phi29, adenovirus], probed with peptide or antibody arrays) are possible, although nucleotide sequence based tagging with oligo array discrimination is favorably convenient, and will therefore be emphasized here. For nucleotide sequence based tagging, the tag sequence is favorably placed such that it is transcribed under the influence of a promoter in the replicon sequence and the appropriate purified RNA polymerase.

A one dimensional array comprising all sequences complementary to all possible tag sequences (or receptors capable of reversibly binding all tag moieties) is formed by the above spatially controlled copolymer array forming methods. Additionally, parallel to the lines of said one dimensional array of said tag sequences, a plurality of lines of oligos of different sequence are formed, preferably during the same patterned synthesis steps. The sequences of the oligos in

each line are chosen to be useful in classifying the fragment incorporated in a given chimera, or the transcript therefrom. The hybridization of each classifying oligo with a sample molecule may be regarded as a positive result from a classification test; each fragment is tested by the full set of classifying oligos (i.e. all lines), such that for n such lines such an array will be capable of discriminating up to 2^n different classification results. Classifying sequences are therefore optimally chosen to have about a 50% chance of probing any given sample fragment or transcript, however large numbers of classifying sequences (lines) may obviate this effort. The essence of this method consists in uniquely identifying a chimera (according to tag identity) and uniquely classifying one terminus of the incorporated sample fragment, which classification information is associated with the identity of the chimera to which it pertains, where the use of copolymer arrays permits the concurrent analysis of large numbers of chimeras or replicons. Correspondence between tag identity and the regions of said lines of said classifying oligos is enforced at the appropriate step with a relief (preferably of elastiomeric composition) of parallel raised lines and trenches, of sufficient spacing to accommodate both the tag probing oligo array elements and the length of the polynucleotide fragments, juxtaposed to the detection oligo array comprising said one dimensional array comprising all sequences complementary to all possible tag sequences and said lines of said classifying oligos, oriented such that said parallel raised lines and trenches are perpendicular to said lines of said classifying oligos.

A library of said chimera, in mixture form, is either transcribed or fragmented, such that for each chimera, a first fragment type comprising a tag sequence and a sufficient length of sample fragment derived sequence is obtained. Such fragments are either produced in labeled form (affinity, fluorescent, radioactive or other label) or otherwise labeled before application to said array. Said lines of said classifying oligos are covered to prevent contact with said first fragment type as a solution containing said first fragment type is contacted with a region of said array comprising said one dimensional array comprising all sequences complementary to all possible tag sequences, to which the molecules of said first fragment type are permitted to hybridize under sufficiently stringent conditions to

ensure high specificity and accuracy of tag identification. Unbound sample molecules are washed away. Said relief is then juxtaposed to said array in said perpendicular orientation. The hybrids formed comprising said sequences complementary to all possible tag sequences are then denatured and a flow of buffer solution is applied along the channels formed by the trenches of said relief and the surface of the substrate of said two dimensional array, and conditions permitting stringent hybridization are then quickly re-established. Thus, the molecules of said first fragment type, which have been confined to particular channels according to the tag sequence which they comprise are swept by said flow across said lines of said classifying oligos and which they thus have the opportunity to hybridize with if and only if they comprise sequences complementary to the sequences of any of said classifying oligos. Unbound molecules are then washed away. Hybridization of sample molecules to said array is then detected by means which correspond to the labeling method used, and the corresponding data favorably recorded by digital computer.

These data are then used to establish the linkage relationship between chimeras according to similarity of classification: two chimeras possibly overlap if the classification of said first fragment type derived from each of them are consistent, i.e. share a sufficiently large number of classifications not shared with an improbably high proportion of chimera. In other words, two sample fragments which partially overlap will be classified similarly according to the hybridization of classifying oligos to the sequences which they share. Combination of data derived from ensembles of such overlap information permit the reconstruction of maps of larger contiguous regions (such as chromosomal material) from which sample fragments derive, and simultaneously yield information about which chimeras (as identified by tag sequences) are derived from particular regions of said maps.

As will be apparent to those skilled in molecular biology and the art of recombinant DNA technology, many different tagging schemes are possible. If care is not taken to ensure that tag sequences differ sufficiently from sample fragment sequence, ambiguous results will be obtained, but the ambiguity of such results will generally be apparent, such that these results may be identified and ignored (e.g. where an improbably high number of classifying oligos are hybridized

within a given channel.) Such ambiguities may be reduced by specifying tag and tag probing sequences by methods similar to those applied to the design of sequencing and PCR primers, i.e. methods which concern the selection of rare sequences and methods which concern the production of degenerate probes (complex mixtures of oligos conforming to some consensus sequence but each hybridizing to different targets having the same complementary consensus sequence) for rare sequences.

Molecules of said first fragment type preferably consist of material from one extremity of the sample fragment incorporated into the respective chimera. This minimizes the degree of overlap between two chimeras classified as contiguous and thus minimizes the number of chimeras necessary to represent the original linear sample, reducing the work necessary for analysis. Note, however, that more redundant information may be obtained by permitting a greater degree of overlap, for example, by producing and then analyzing longer molecules of said first fragment type. Thus, two extreme cases are possible: said first fragment type comprising most all of a chimera; and said first fragment type consisting of a minimal length of sequence to permit useful classification. In practice, tradeoffs exist between these extremes, and intermediate cases are probably preferable according to the specific library and sample.

As will be apparent, each of said molecules of said first fragment type must comprise said tag sequence and sequence from said original sample from which said library was derived. Where said molecules of said first fragment type are limited to one terminus of the sample fragment sequence (i.e. one sample fragment sequence region adjacent to one arm of the replicon vector used to construct the library), the method of preparation of said first fragment type must ensure that the tag sequence associated with one terminus may be correlated with the tag sequence associated with the other terminus. At least two instances conforming to this requirement are possible.

In a first instance, said molecules of said first fragment type may be prepared by restricting aliquots of the library of said chimera differently. Here, a the vector from which said library was prepared comprises a first unique restriction enzyme site, which may be chosen according to infrequent occurrence in the subject genome or original sample, on one side of a tag sequence, and a second unique restriction

enzyme site, which may be chosen according to infrequent occurrence in the subject genome or original sample, on the other side of a tag sequence. In this case, a first aliquot is subjected to restriction digestion with the enzyme specific for said first unique restriction enzyme site, and a second aliquot is subjected to restriction digestion with the enzyme specific for said second unique enzyme site. Additionally, fragments may be shortened by any convenient method such as shearing, random nuclease treatment, etc., to restrict the length of polynucleotide fragments captured by tag probe oligos and subsequently analyzed to convenient lengths. Each aliquot is analyzed separately according to this aspect of the present invention, and results are used to correlate similarly classed fragment sequences found in said first aliquot with those found in said second aliquot.

In a second instance, each molecule of said vector from which said library was prepared comprises two different, unique tag sequences, each located near a terminus of said molecule, i.e. near the locus to which a sample fragment is ligated. Thus, each tag sequence corresponds to a terminus of a sample fragment in the library constructed with said vector. Molecules of said first fragment type having exactly one of said two different, unique tag sequences are produced by known art methods which will be related in consideration to the design of said vector (e.g. restriction digestion, transcription from an artificial promoter, etc., according to corresponding vector features in relation to said two different, unique tags and the inserted said sample fragment.) Said molecules of said first fragment type having exactly one of said two different, unique tag sequences are analyzed according to the above library analysis methods, probing in each case with the members of only one tag probe set. Because each terminus is associated with a different tag in each clone, the tag from one terminus must be correlated with the tag of the other terminus. This second correlation step is accomplished by the same method, binding chimera or fragments comprising the vector termini with a one dimensional tag probing oligo array complementary to the first tag set, where lines of different classifying oligos in the array are replaced with lines of different tag probing oligos, corresponding to those which bind tag sequences of the second tag set.

The foregoing embodiments and examples have been presented for illustration rather than limitation, with the many possible variations, changes and alternative embodiments which will be obvious to those of ordinary skill in the relevant arts included within the scope and spirit of the present invention. Thus, the scope and the breadth of the present invention is intended to be defined by the appended claims rather than the foregoing description.

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whereforth, I claim:

Claims:

1. A process for the fabrication of microscale or nanoscale devices comprising the use of replicated patterned relief surfaces and a mask forming step selected from the group consisting of: multilayer vetoing of self-assembling monolayer patterns on metallic films; polymerization of resist from patterned initiator compound regions; casting of polymeric resist patterns on surfaces; injection molding of resist precursors; embossing of a preformed resist film followed by a mild etch to eliminate reduced thickness regions; curing of prepolymer solutions wetting underlying patterns.

2. A process according to claim 1 further comprising the incorporation of alignment patterns into said replicated patterned relief surfaces and the surfaces to which these are juxtaposed, where said alignment patterns are selected from the group consisting of: patterns which cause the appearance of Moiré patterns when overlapped; interlocking surface relief patterns.

3. A process for the patterning of three or more distinct affinity species or reactants serving as precursors thereof on a surface comprising one or more steps of contacting said surface with one or more micropatterned relief surfaces.

4. A method according to claim 3 for the spatially controlled synthesis of copolymer sequences on solid surfaces comprising the use of relief surfaces to effect or prevent the physical contact of reactants or reagents with regions of said solid surfaces.

5. A method for the formation of molecular layers having spatially predetermined compositional pattern, comprising one or more steps of associating terminal affinity group functionalized molecules with complementary affinity groups patterned onto surfaces or other such molecular layers.

6. A method for the replication of compositional patterns of molecular layers produced according to claim 5.

7. A method for the positionally controlled synthesis of molecules or molecular complexes according to positional constraint design rules and comprising the use of positioning means which directly or indirectly bind reactant molecules, reactant precursor molecules, catalysts, enzymes, abzymes, ribozymes, deprotection reagent molecules, activator reagent molecules or protecting reagents, and position these relative to reactive groups situated on a workpiece or workpiece precursor comprising one or more molecules.

8. A method according to claim 7 where said positioning means comprises a microfabricated array.

9. A method for the spatially ordered dissection of genetic material comprising the immobilization of said genetic material to a predefined region of a surface.

10. A method according to claim 9 comprising the use of micropatterned surfaces.

11. A method for the determination of the ordering of clones or polynucleotide maps from a polynucleotide library comprising the use of one or more oligonucleotide or polynucleotide arrays.

12. A method for the fabrication and replication of data patterns on surfaces for read out by data readout means comprising scanned probes comprising the use of one or more patterned relief surfaces.

13. Method and means for readout of data patterns and surfaces prepared according to claim 12 comprising a charge coupled device and actuation means for translating said surfaces relative to said charge coupled device.

14. A method according to claim 11 further comprising the use of an elastiomeric relief comprising raised parallel stripes with microscale features.

15. A process for the patterning of two or more distinct materials or precursors thereof on a porous surface comprising one or

more steps of contacting said surface with one or more micropatterned relief surfaces each of which is coated with a suspension of one of said distinct materials.

16. A method according to claim 15 further comprising an annealing step.

17. A method for the fabrication comprising the steps of:

(a) creating one or more elastiomeric microreliefs;

(b) coating said elastiomeric microrelief with a material from which an article is to be fabricated;

(c) juxtaposing said elastiomeric microrelief of step (b) to a surface;

(d) causing said material from which an article is to be fabricated to polymerize or harden or set;

(e) removing said elastiomeric microrelief;

(f) forming or molding a sacrificial material over the surface produced in step (d) and causing said sacrificial material to polymerize or harden or set if necessary;

(g) repeating steps (b) through (f) until the article under fabrication is complete; and,

(h) removing said sacrificial material added in any step (f).

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(57) Abstract

Methods for the formation and replication of patterns are disclosed, with objectives including economical microfabrication and nanofabrication, suitable for both low and high volume production. A patterned relief is formed on a surface by various techniques, which is then replicated on daughter surfaces. Methods for the reduction or elimination of defects are included. Various compositions may be patterned to address a wide range of applications. Spatial control over copolymer synthesis, synthesis of patterned molecular monolayers and multilayers, fabrication of microelectronic, microelectromechanical and microfluidic devices may be effected by the methods of the present invention. Various articles of manufacture and uses thereof, including improved methods and means for genome analysis, are further disclosed.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB96/00912

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :101/450.1, 454, 458, 461, 463.1; 435/6; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 101/450.1, 454, 458, 461, 463.1; 435/6; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS, INPADOC
search terms: lithography, relief, array, support, peptide, oligonucleotide, combinatorial synthesis, hybridization

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,981,783 A (AUGENLICHT, L.) 01 January 1991, especially figure 6, columns 6 and 13.	3-16
X	US 4,242,378 A (ARAI, Y.) 30 December 1980, column 1.	1, 2, 17
X	MILLMAN, J. 'Integrated Circuits: Fabrication and Characteristics.' In: Microelectronics: Digital and Analog Circuits and Systems. Edited by S. W. Director. New York: McGraw-Hill Book Company. 1979, chapter 4, pages 91-119, especially section 4-4, pages 98-100.	1, 2, 17
X	ABBOTT et al. Using Micromachining, Molecular Self-Assembly, and Wet Etching to Fabricate 0.1-1-μm-Scale Structures of Gold and Silicon. Chem. Mater. May 1994, Vol. 6, No. 5, pages 596-602, especially pages 597-598.	1, 2, 17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB96/00912

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

B41F 1/18, 7/00; B41N 1/00, 3/00; B41M 5/00; C12Q 1/68; C07H 21/02

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1, 2, and 17, drawn to processes of fabrication of microscale or nanoscale devices.
Group II, claim(s) 3-8, 12, 13, 15, and 16, drawn to methods of pattern formation and combinatorial synthesis.
Group III, claim(s) 9-11 and 14, drawn to methods of ordering genetic material.

The inventions listed as Groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The fabrication processes of Group I do not necessarily involve affinity species or genetic material and the devices fabricated by said methods can be used for purposes other than dissection of genetic material. Likewise, the methods of patterning affinity species and data pattern readout of Group II are not limited to genetic materials as is Group III and do not involve analysis of a nucleic acid containing sample.